

# Chapter 2

## Strategies for Altering Plant Traits Using Virus-Induced Gene Silencing Technologies

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### Abstract

The rapid progress in genome sequencing and transcriptome analysis in model and crop plants has made possible the identification of a vast number of genes potentially associated with economically important complex traits. The ultimate goal is to assign functions to these genes by using forward and reverse genetic screens. Plant viruses have been developed for virus-induced gene silencing (VIGS) to generate rapid gene knockdown phenotypes in numerous plant species. To fulfill its potential for high-throughput phenomics, it is of prime importance to ensure that parameters conditioning the VIGS response, i.e., plant–virus interactions and associated loss-of-function screens, are “fit for purpose” and optimized to unequivocally conclude the role of a gene of interest in relation to a given trait. This chapter will review and discuss the different strategies used for the development of VIGS-based phenomics in model and crop species.

**Key words** Plant functional genomics, Virus-induced gene silencing, RNAi, Forward and reverse screens, Model plants, Crops

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### 1 Introduction

Rapid progress in genome sequencing and transcriptome analysis using Next Generation Sequencing (NGS) technologies and microarray platforms are revolutionizing plant science. Genetically complex plant species are receiving unprecedented interest in sequencing their genomes with the ultimate aim to link genotype to phenotype for economically important traits [1]. Gene function characterization by modifying gene expression and its phenotype is widely considered the main bottleneck of the postgenomic era [2]. In the past two decades, plant viruses have become instrumental in studying plant–pathogen interactions and understanding the multifaceted nature of plant resistance mechanisms. The genetic engineering of viruses has opened up a wide range of applications [3] including the characterization of virus-encoded gene functions and monitoring their movement *in planta* using a fluorescent protein tag [4]. Scientists have exploited the properties of plant viruses as

episomal overexpression vectors to develop functional genomic platforms, first as gain-of-function assays, by expressing functional full-length cDNAs of endogenous or nonendogenous proteins and studying their effect *in planta* [5–8], and further as loss-of-function assays [9, 10] by switching off host gene expression.

The first example of virus-induced gene silencing (VIGS), defined as the induction of a loss-of-function phenotype, was first reported back in 1995 [9]. In this first VIGS system, a *Tobacco mosaic virus* (TMV) expression vector was used to knock down genes involved in the carotenoid biosynthetic pathway by expressing a cDNA fragment of *phytoene desaturase* in antisense orientation [9]. This first example of so-called cytoplasmic inhibition of gene expression illustrated the potential of virus vectors in rapidly inducing (within 2–3 weeks after inoculation) a loss-of-function phenotype by expressing antisense virus-encoded transcripts and opened up avenues for the rapid assessment of gene function in plants. Since then, an ever growing number of applications using different virus species or virus-derived episomal genetic elements have been reported. This has led to the expansion of VIGS to numerous plant species, with the development of novel loss-of-function screens, exemplifying the strong potential of this approach for functional genomics in genetically complex plants [11].

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## 2 Mechanisms and Dynamics of Virus-Induced Gene Silencing in Plants

VIGS is a manifestation of an endogenous RNA-mediated defense mechanism (referred to as RNA interference or RNAi) that targets a wide range of genetic elements, including transposons, improperly matured RNAs, and viruses. During this process, double-stranded (ds)RNA molecules are recognized by RNase III-like enzyme, namely, Dicer-like endonuclease (DCL) and cleaved into small interfering (si)RNAs. Single-stranded siRNAs will be incorporated into the RNA-induced Silencing Complex (RISC) involving Argonaute, and other associated proteins will recognize and guide an homology-dependent degradation of the homologous target viral RNA. The viral genome will therefore be the trigger and the target of RNAi leading to the degradation of the viral RNA [12]. Introduction of a plant cDNA fragment into the viral genome will redirect the RNAi response to promote the degradation of host mRNAs and inhibit corresponding gene expression. While the majority of plant-infecting viruses have a (+)ssRNA genome, DNA viruses such as Caulimoviruses and Geminiviruses are both inducers and targets of RNAi. The formation of aberrant dsRNA in these cases is believed to originate either from RNA replicative intermediate by pairing between (–) and (+)ssRNA strands during replication of (+)ssRNA or overlap of sense and antisense RNAs from bidirectional promoters or from folded secondary structures of abundant viral RNAs [13].

Systemic movement of siRNA has been reported during virus infection and in transgene-induced silencing [14]; in the case of VIGS, the RNAi response to endogenous genes is closely associated with cell layers supporting virus replication. Monitoring the systemic silencing response generated by knock down of the *Phytoene desaturase* (*PDS*) or *Sulphur* (*SU*) gene provides a robust means to map the distribution of the RNAi response [9, 10, 15]. Significant variations in the efficiency of the silencing response do occur between viruses and between plant species or closely related ecotypes or cultivars [16] with fluctuations (cycles of fading and reappearance in emerging leaves [17]) to a sustained, albeit often relatively weak, silencing response that can persist through seed stage in the progeny [18, 19] sometimes up to 2 years after the initial inoculation [20]. RNAi acts as a counter-selective mechanism hampering virus accumulation and ultimately VIGS efficiency. Viruses have developed counter-defense mechanisms to evade RNAi, and some virulence factors of plant viruses act as suppressors of RNAi [21]. The complex dynamics of host RNAi and virus counter-defense mechanisms might explain the nonuniformity of the silencing phenotype observed.

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### 3 VIGS Systems

#### 3.1 Viruses, Satellite-Associated Molecules, and Virus- Encoded Genes

Originally, the first examples of virus-induced gene silencing relied on a limited number of (+)ssRNA virus vectors that were initially developed and designed to express full-length cDNAs. These included TMV, *Potato virus X* (PVX), and *Tobacco rattle virus* (TRV) [9, 10, 22]. Their use as silencing vectors was exemplified by using cDNA fragments in sense or antisense orientation to trigger the silencing of the endogenous reporter gene, *PDS*. This approach provided a landmark for the development and improvement of these early VIGS platforms and the development of new VIGS vectors. Exploiting the patterns of the systemic movement of a virus offers possibilities to promote or enhance silencing in specific areas of the plant. This was demonstrated using a modified TRV-VIGS vector that retains its helper protein, 2b, required for nematode transmission and provided a means to trigger robust silencing in root tissues [23]. However, not all ss(+) RNA viruses are amenable to generating a robust silencing response, as some virus genera such as potyviruses encode potent silencing-suppressing proteins which prevent their use as a silencing platform. Other VIGS systems relied on the use of satellite RNA of a replicating helper TMV virus to deliver dsRNA in infected plants. The advantage of this approach is in the uncoupling of virus replication function from its silencing induction function mediated by the satellite RNA [24]. This approach triggered strong VIGS knockdown phenotypes in its host, *Nicotiana tabacum* [24].

While size constraints in packaging their encapsidated genomic DNA have hampered the use of DNA viruses as expression platforms of full-length cDNAs, geminiviruses (ssDNA) have proven to be an efficient VIGS platform (*Tomato golden mosaic virus* TGMV, *Cauliflower leaf curl virus* CaLCuV, *African cassava mosaic virus* ACMV) in a wide variety of plant species such as *Nicotiana benthamiana* and *Arabidopsis thaliana* (Table 1). As opposed to RNA viruses whose replication cycle occurs in the cytoplasm, DNA viruses replicate in the nuclei and trigger homology-dependent degradation of target transcripts [15]. Silencing of several genes has been reported using a TGMV VIGS vector, including in meristematic cell layers from which most plant viruses are excluded [15]. As for RNA viruses, satellite ssDNA molecules can be transformed as VIGS vectors [25]. Further, *Rice tungro bacilliform virus* (RTBV), a dsDNA pararetrovirus from the *Caulimoviridae* family, was developed as a VIGS vector for rice [26], illustrating the potential of dsDNA viruses to trigger an efficient silencing response in this economically important host.

### 3.2 Nature of the Elicitor of the Silencing Response

cDNA fragments of different lengths, mainly in antisense orientation, have been used as elicitors of the silencing response. Previous studies have reported that cDNA length affects the silencing response. The *barley stripe mosaic virus* (BSMV) VIGS vector can induce silencing with fragments ranging from 128 to 584 nt with comparable efficiency, suggesting that insert size does not always correlate with increased silencing response [18] in this system. In contrast, BSMV accumulation was affected in constructs harboring larger inserts (i.e., 584 nt in length), and the silencing response lasted to the next generation with BSMV constructs harboring smaller inserts (80–125 nt). Studies on a PVX-VIGS vector demonstrated that fragments as small as 33 nt in length can trigger significant silencing of the *PDS* gene in *N. benthamiana* [27].

While inverted repeats have proven to be a potent trigger of PTGS [28] in transgenic plants, hairpin RNA (hpRNA), folding back as dsRNA upon transcription, has been found to generate a strong silencing response in some VIGS systems (TMV, BSMV, TYMV) [29, 30]. This approach offers the possibility of cloning smaller fragments, i.e., from 40 nt up to 60–80 nt in length per repeat, with the view of narrowing the size of the target RNA fragment. The benefits of smaller sized RNA and dsRNA lies in minimizing off-target effects by selecting smaller transcript regions that are unique to the gene family and avoiding unwanted silencing of closely related gene families [31].

Further refinement of VIGS systems involves the overexpression of artificial micro (ami)RNAs from a virus vector [32]. In this approach, the authors used the properties of miRNAs (small non-coding RNAs of 18–25 nt in length) to regulate gene expression by promoting target mRNA degradation. amiRNAs can be

**Table 1**  
**Examples of VIGS systems developed in model and crop species**

<b>Virus genome composition</b>	<b>Genus</b>	<b>Virus</b>	<b>Plant species silenced</b>	<b>Tissue silenced</b>	<b>Cloning</b>	<b>Delivery</b>	<b>Applications</b>	<b>References</b>
(+) ss RNA	Tobravirus	Tobacco rattle virus (TRV)	<i>N. benthamiana</i> , <i>Solanum esculentum</i> , <i>Solanum tuberosum</i> , <i>Solanum chacoense</i> , <i>Solanum nigrum</i> , <i>Petunia hybrida</i> , <i>Arabidopsis thaliana</i> , <i>Phalaenopsis equestris</i> , <i>Gossypium hirsutum</i> , <i>Fragaria ananassa</i> , <i>Papaver somniferum</i>	Leaves Flowers Fruits Seeds Meristems	Multiple Cloning Site (MCS) GATEWAY recombination Ligation-Independent Cloning	Infectious RNA mechanical (wound) inoculation Agroinfiltration Agrodrench	Plant–pathogen interactions: host resistance and susceptibility to viruses, bacteria, fungi, nematodes; elicitor-mediated response; plant–symbionts interactions; plant–herbivores interactions; high-throughput forward genetic cDNA screening of genes associated with nonhost resistance, Agrobacterium-mediated transformation, host-induced gene silencing to nematodes; development	[10, 23, 36, 37, 54, 57–64]

(continued)

**Table 1**  
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<b>Virus genome composition</b>	<b>Genus</b>	<b>Virus</b>	<b>Plant species silenced</b>	<b>Tissue silenced</b>	<b>Cloning</b>	<b>Delivery</b>	<b>Applications</b>	<b>References</b>
		Pea early browning virus (PEBV)	<i>Pisum sativum</i> , <i>Medicago truncatula</i> , <i>Lathyrus odorata</i>	Leaves Roots	MCS	Infectious RNA	Plant–symbiont interactions	[65]
	Potexvirus	Potato virus X (PVX)	<i>N. benthamiana</i> , <i>Solanum tuberosum</i> , <i>Solanum bulbocastanum</i>	Leaves Tubers In vitro grown plantlets Microtubers	MCS	Infectious RNA Agroinfiltration Agroinoculation colonies	Forward genetic cDNA library screening of genes associated with hypersensitive response, development.	[46, 66]
	Comovirus	Bean pod mottle virus (BPMV)	<i>Glycine max</i> , <i>Phaseolus vulgaris</i>	Leaves Roots	MCS	Biolistic	Susceptibility to virus, resistance to pathogens; development	[67, 68]
	Hordeivirus	Barley stripe mosaic virus (BSMV)	<i>Hordeum vulgare</i> , <i>Triticum aestivum</i> , <i>Zea mays</i> , <i>Avena sativa</i> , <i>Avena strigosa</i> , <i>Brachypodium distachyon</i> , <i>Zingiber officinale</i> , <i>N. benthamiana</i>	Leaves Seeds Roots	MCS, Ligation-independent cloning	Infectious RNA Agroinfiltration Biolistic	Plant–pathogen interactions (host, nonhost resistance, susceptibility), host-induced gene silencing to phytopathogenic fungi, plant development	[16, 19, 44, 55, 69–73]

Bromovirus	Brome mosaic virus (BMV)	<i>Oryza sativa</i> , <i>Hordeum vulgare</i> , <i>Zea mays</i> , <i>Setaria italica</i> , <i>Sorghum bicolor</i>	Leaves	MCS	Infectious RNA Agroinfiltration	Development, Plant–Pathogen Interactions	[74, 75]
Cheravirus	Apple latent spherical virus (ALSV)	<i>N. tabacum</i> , <i>N. occidentalis</i> , <i>N. glutinosa</i> , <i>N. benthamiana</i> , <i>S. esculentum</i> , <i>A. thaliana</i> , <i>Cucumis melo</i> , <i>C. sativus</i> , <i>Cucurbita pepo</i> , <i>Citrullus lanatus</i> , <i>Luffa cylindrica</i> , <i>Lagenaria siceraria</i> , <i>Glycine max</i> , <i>Pisum sativum</i> , <i>Vigna angularis</i> , <i>Vigna unguiculata</i>	Leaves	MCS	Wound inoculation DNA constructs followed by passage inoculation of infectious sap	Development	[76, 77]
Tobamovirus—RNA satellite virus	TMV—Satellite tobacco mosaic virus (STMV)	<i>N. tabacum</i>	Leaves Flowers	MCS	Infectious RNA Agroinfiltration	Development	[24]
ssDNA Begomovirus	Tomato golden mosaic virus (TGMV)	<i>N. benthamiana</i>	Leaves Meristems Flowers	MCS	Biolistic	Development	[78, 79]

(continued)

Table 1  
(continued)

Virus genome composition	Genus	Virus	Plant species silenced	Tissue silenced	Cloning	Delivery	Applications	References
		Tomato yellow leaf curl china necrotic virus (TYLCCNV)—satellite DNA vector	<i>S. esculentum</i> , <i>N. glutinosa</i> , <i>N. tabacum</i>	Leaves Flowers Meristems	MCS	Agroinfiltration	Development	[25, 80]
		Beet curly top virus (BCTV)	<i>Spinacia oleracea</i> , <i>S. esculentum</i>	Leaves Meristems	MCS	Biolistic Agroinfiltration	Development	[81]
		Cabbage leaf curl virus (CaLCuV)	<i>A. thaliana</i>	Leaves	MCS	Biolistic	Development	[79]
dsDNA	Caulimoviridae—Tungrovirus	Rice tungro bacilliform virus (RTBV)	<i>Oryza sativa</i>	Leaves	MCS	Agroinfiltration	Development	[26]



designed to silence either single or multiple target genes [33]. This approach, termed MIR-VIGS, was successfully used to knock down a range of endogenous genes and, in spite of the small size of the silencing trigger, compared favorably to classical siRNA-derived VIGS constructs using larger fragments [32].

### 3.3 Strategies Used to Deliver VIGS Vectors

The generation of infectious RNA from (+)ssRNA-derived constructs required an in vitro transcription step from a linearized plasmid template driven by a T7 promoter to produce infectious transcripts of either single genomic RNA (PVX, TMV) or multiple genomic RNAs (TRV, BSMV) (Table 1). While being widely used, this approach can be onerous as VIGS screens require the generation of a sufficient amount of templates (even more so for multipartite virus genomes) to inoculate a suitable number of biological replicates in several independent inoculation experiments of control and target plants. Alternatives were sought and, when possible, infectious sap can be used from this initial infection event to produce a larger bulk of infectious VIGS constructs. Further refinements were brought using biologically delivered plasmids which generate infectious viral genomic RNA *in planta* through CaMV 35S promoter-driven transcription and linearization by a self-cleaving ribozyme in their 3'-end viral RNA. Such an approach is currently used to deliver a BSMV-VIGS vector in monocot hosts ([34], Table 1). A robust alternative relies on *Agrobacterium tumefaciens* (agroinoculation) harboring the virus genome within the T-DNA of a Ti plasmid which will be transferred into the genome of the plant. This approach was successfully used for a range of VIGS vectors such as PVX, TRV, and PEBV (Table 1). Diverse methods of agroinoculation have been reported (Table 1). Agroinoculation offers many advantages including: (1) reducing the cost of generation of infectious viruses, (2) infection of plants at an early developmental stage before full leaf development using agrobacteria suspension for infiltration of root tissues (termed “agrodrench,” [35]), and (3) infiltration of agrobacteria into specific tissues such as fruits by syringe-mediated agroinfiltration (Table 1). Availability of TRV vectors with ligation-independent cloning systems with a high efficacy of cloning [36] simplified the cloning step and made VIGS vectors suitable for high-throughput reverse screening. These advances made it possible to study genes regulating fruit development and response to numerous biotic and abiotic stresses, alleviating the initial drawbacks of VIGS which now can be applied to most, if not all, plant developmental stages (Table 1).

## 4 Applications of VIGS

### 4.1 *N. benthamiana*: The Model Plant of Phenomics

The robustness of a VIGS screen requires not only a suitable virus-silencing vector but also a host that fulfills at least some of the following criteria: (1) tolerance of virus accumulation and systemic movement in most organs, (2) low symptomatology to virus

infection to minimize unwanted effects that could interfere with the silencing screen, and (3) induction of a robust silencing response with acceptable intensity (i.e., observable silencing phenotype and decrease of target RNA levels), coverage, and duration.

Albeit VIGS vectors have been developed and demonstrated to trigger gene silencing in *A. thaliana* [30, 37], *N. benthamiana* has become the host of choice for VIGS-based functional genomics (also termed “phenomics” [2]) due to its early adoption by the plant virology community for its ability to support infection by many virus species [38]. *N. benthamiana*’s amenability to Agrobacteria-based transient gene expression and susceptibility to various pathogens and pests (bacteria, fungi, viruses, oomycetes, nematodes, and insects) allowed scientists to develop many VIGS-based screens to unravel the molecular nature of many types of plant–pathogen interactions from elicitor-based response, hypersensitive response, and host and nonhost resistance in a range of tissues and organs (Table 1) [11].

The development and recent completion of genome sequencing projects for a number of related economically important solanaceous crops such as tomato [39] and potato [40] have highlighted the need for the scientific community to use *N. benthamiana* as a surrogate host. Indeed, *N. benthamiana* is more amenable for robust VIGS-based phenomics of an increasingly large number of genes from related solanaceous crops that bear sufficient sequence homologies for heterologous silencing (Table 1). The draft genome of *N. benthamiana* (size of 2.6 Gb with 16,000 unigenes deposited in GenBank) has recently been published [40, 41]. These resources will contribute not only to the facilitation of cDNA cloning but also to the design of more refined VIGS constructs to target single or multiple genes within a family by using a cDNA silencing trigger that minimizes off-target effects [31]. Together with the availability of microarrays for transcriptome profiling, EST database, transient and stable transformation protocols, transgenic marker lines and VIGS libraries strengthen *N. benthamiana* as a model plant for phenomics.

## 4.2 VIGS-Based Phenomics in Crop Species

The successive improvements of VIGS vectors and their mode of delivery in host plants have considerably widened the use of VIGS as a versatile gene knockdown platform. One of the most important milestones in VIGS-based phenomics is its expansion from model plants to crops for the study of unique metabolic or developmental pathways. VIGS systems have been implemented for many plant species with economical interest as main sources of food worldwide (such as rice, wheat, maize, barley, tomato, potato, soybean, pea, bean, and strawberry), secondary metabolites (alkaloids in tobacco and poppy), floral morphogenesis in ornamentals (*Solanaceae* and *Orchidaceae*), and fibers (cotton) in very diverse plant species from dicotyledons, monocotyledons, and woody perennials (apple, pear, and grapevine) (Table 1, [11, 42, 43]).

VIGS screens were developed to study organ development and biosynthetic pathways in most plant tissues (leaf, root, flower, tubers, and seeds) at early or late developmental stages in progeny plants and to study most types of plant–pathogen interactions (Table 1). So far, due to their broad host range, TRV and BSMV VIGS vectors have emerged as generic VIGS systems for many crop species, including genetically complex hexaploid (wheat) or octaploid (strawberry) species for which a mutagenesis approach remains a huge challenge [44, 45].

### 4.3 VIGS-Based Forward Genetics Screens

While VIGS has been mainly used as a reverse genetics approach to characterize defined target genes, high-throughput forward genetics screens have been developed by cloning normalized cDNA libraries into VIGS vectors and screening for a phenotype of interest. The potential of this approach has been exemplified by screening about 5,000 cDNAs for the suppression of localized cell death associated with the hypersensitive response (HR) during resistance to the bacterial phytopathogen *Pseudomonas syringae* using a PVX VIGS vector [46]. Among the six candidates that suppressed HR, the authors identified Heat Shock Protein 90 (HSP90) as a cochaperone of disease resistance proteins whose knockdown resulted in the suppression of HR cell death [46]. The authors estimated that this forward screen might have covered about 10 % of the *N. benthamiana* transcriptome (~2,500 genes). In a separate study, a TRV-VIGS vector was used to screen 1,500 cDNAs for their ability to alter cell death development in *N. benthamiana*. This led to the identification of *Beclin1* whose knockdown phenotype resulted in uncontrolled cell death, thereby defining *Beclin1* as a key regulator of autophagy-associated pathways by restricting HR cell death to the initial infection site [47]. Since then, other examples of VIGS-based forward screening of cDNA libraries have been reported (Table 1).

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## 5 Current Limitations of VIGS

The properties of virus-derived expression vectors offer many advantages over conventional stable transformation, allowing rapid functional studies on plants that are recalcitrant to transient or stable transformation. As the intensity and coverage of the VIGS response vary between plants and experiments, it is therefore important to ensure that a gene in a specific tissue is efficiently silenced and associated to the phenotype of interest, highlighting the influence of environmental conditions in the development and sustainability of the VIGS response [48, 49]. Cosilencing of reporter genes together with a gene of interest from the same VIGS vector has been reported and offers a means to identify plants or tissues that undergo silencing [37]. However, the choice of reporter gene is crucial because steady state RNA and protein

turnover vary between genes and, in the case of *GFP* transgene systemic noncell autonomous silencing, has been reported in *N. benthamiana* [50] and might not represent faithfully the extent of the silencing response of other endogenous genes of interest in this system. Recently, other transgenic reporter systems were described to visualize silenced areas in tomato fruits [51].

While efforts are made to select a VIGS plant system that does not display strong symptoms of viral infection, the virus life cycle induces substantial cellular modifications from the host machinery to perform viral replication and movement that will impact plant metabolism [52]. VIGS screens require careful selection of varieties/ecotypes that tolerate virus accumulation and suitable control plants (i.e., virus infected with closely related constructs that trigger and do not trigger VIGS) to get an accurate representation of the phenotype associated with the knockdown of the selected gene.

The influence of the genetic background of the host is likely to impact the robustness of virus accumulation and, concomitantly, the VIGS response generated. *N. benthamiana* was shown to lack a RNA-dependent RNA polymerase 1 (*RDR1*) activity which is a component of the antiviral defense mechanisms making the plant more susceptible to viruses [53]. Further reverse engineering of selected plants or screening for ecotypes that are deficient in RDR activity might prove to be an efficient means to engineer recalcitrant host plants more amenable to VIGS-based approaches.

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## 6 Novel Approaches

### 6.1 Hijacking the microRNA

**Pathway: mirVIGS**

As described earlier in Subheading 3.2, micro RNAs (miRNA) can be used to promote specific gene silencing. The authors have demonstrated that artificial miRNAs can be designed and expressed from a cabbage leaf-curl geminivirus (*CaLCV*) to silence the expression of several endogenous reporter genes (*PDS*, *SU*), flower development (*CLA*), and the genes involved in *N*-mediated resistance to TMV (*SGT1*) [32]. Using artificial miRNAs (MIR VIGS) offers a means to design a VIGS construct which minimizes off-target effects and ensures that a phenotype is associated with the selected gene target. Moreover, this approach opens up a way to study miRNA function rapidly and a powerful screening method to engineer stable knockdown assays.

### 6.2 Coupling VIGS with Other Omics

**Platforms: Reverse Engineering of Metabolic Pathways**

The development of a visually traceable VIGS response was recently reported in tomato fruit [50]. The overexpression of *Antirrhinum majus Delia* and *Rosea1* transcription factors in tomato yielded anthocyanin-rich purple tomato fruits, in combination with a TRV tandem VIGS vector cosilencing *Delila/Rosea1* together with the gene of interest. The recovery of red-colored segments of fruits provided a convenient means to identify the silenced area, which,

coupled with metabolic (volatile) profiling, exemplified the potential of this approach to assess the impact of gene knockdown on the metabolome and a means to map regulatory networks [51].

### **6.3 Host-Induced Gene Silencing From Invading Microorganisms**

While most of the applications of VIGS focus on the alteration of the phenotype of the plant host, recent studies have demonstrated that gene silencing generated in plants can target invading microorganisms.

This was exemplified using a TRV-VIGS vector to generate dsRNA into the feeding cells and to mediate gene silencing to invading root-knot nematodes [54]. Interestingly, the knockdown of the targeted genes was observed in the progeny of the feeding nematodes, suggesting that this approach could be used for the functional analysis of genes involved in the early development of nematodes *in planta*. One of the main drawbacks of this approach is the heterogeneity in RNAi efficiency between inoculated plants which yet prevent its use for the high-throughput functional analysis of selected nematode genes.

Using transgenic plants and the BSMV VIGS system, Nowara et al. [55] demonstrated that RNA interference with gene expression of the biotrophic fungus *Blumeria graminis* (powdery mildew) in barley and wheat was effective and inhibited *Blumeria* colonization. In this approach, termed Host-induced Gene Silencing (HIGS), the authors triggered RNAi of the *Blumeria* avirulence gene *Avr10* whose knockdown promoted fungal growth in barley cultivars harboring the matching *Mla10* resistance gene. Since then, HIGS was demonstrated to knock down the expression of three potential pathogenicity genes from the wheat rust fungus *Puccinia triticina* which resulted in a suppressed disease phenotype [56].

Host-induced knockdown of invading pathogen genes has a strong potential to expand functional genomics to invading pathogens and to develop an efficient means of protecting plants against pathogens and pests.

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## **7 Perspectives**

The strength of VIGS lies in its versatility and rapidity of altering gene expression in a range of plant species within a few weeks from cloning to visual assessment of the knockdown phenotype *in vivo*. VIGS-based phenomics have greatly contributed to the recent advances in many areas of plant science. In turn, the development of VIGS phenomics has benefited from the knowledge of RNA regulatory pathways that shape the molecular and cellular nature of plant-virus interactions and plant developmental pathways. The ongoing development of new virus-derived expression vectors and novel phenomics screens will undoubtedly broaden our knowledge

of gene function in many plant species. Getting further insight on these key mechanisms in model and genetically complex crop species will allow the scientific community to get a broader understanding of the regulation of complex traits and ultimately the development of sustainable strategies for crop production and protection against pathogens and pests.

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