Virus Induced Gene Silencing – A Tool For Gene Function Analysis In Plants

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Abstract- *Virus Induced Gene Silencing is caused by an RNA-mediated defense mechanism against viruses in plants. This mechanism has been exploited to study the gene function in plants. Virus Induced Gene Silencing technique involves the use of recombinant viruses to knock-down expression of endogenous genes. The targeted down regulation of a particular gene transcripts by Virus Induced Gene Silencing made it a powerful tool in gene function analysis. The phytoene desaturase and chalcone synthase gene can be used as reporter genes in viral vector. VIGS avoids plant transformation and allows rapid comparison of gene function between species in different genetic backgrounds. These properties make Virus Induced Gene Silencing a superior tool in plant gene function analysis.*

Keywords- Virus Induced Gene Silencing, functional analysis, gene suppression, viral vectors

I. INTODUCTION

In 1866, Gregor Mendel published his work in a paper entitled “Experiments on plant hybridization” in which he explained that there is something responsible for the production of phenotype. He called that something as “factors” which was later known to be “genes”. Since then many plant biologists have made several effort to relate the phenotype of an organism to genotype. The two main approaches to study gene functions are (1) Forward genetics (2) Reverse genetics.

A. Forward genetics

Forward genetics is an approach that encompasses several means of identifying the gene or set of genes that are responsible for a particular phenotype within an organism. Initially, this entailed the generation of random mutations in an organism, often through radiation, and then through a series of breeding of subsequent generations, isolating individuals with an aberrant phenotype. Forward genetics can be thought of as a counter to reverse genetics, which seeks to alter genes in order to illuminate their multiple phenotypes. By the classical genetics approach, a researcher would then locate (map) the gene on its chromosome by crossbreeding with individuals that carry other unusual traits and collecting statistics on how frequently the two traits are inherited together. Classical geneticists would have used phenotypic traits to map the new mutant alleles [1].

Disadvantages of forward genetics

Saturation mutagenesis within classical experiments was used to define sets of genes that were a bare minimum for the appearance of specific phenotypes. However, such initial screens were either incomplete as they were missing redundant loci and epigenetic effects, and such screens were difficult to undertake for certain phenotypes that lack directly measurable phenotypes.

B. Reverse genetics

Reverse genetics is an approach to discovering the function of a gene by analyzing the phenotypic effects of specific gene sequences obtained by DNA sequencing. This investigative process proceeds in the opposite direction of so-called forward genetic screens of classical genetics. In simple terms, forward genetics seeks to find the genetic basis of a phenotype or trait while reverse genetics seeks to find what phenotypes arise as a result of particular genes. Most reverse genetics approaches described in plants to date rely on posttranscriptional gene silencing [2]. Knocking out genes is the most frequently used strategy of reverse genetics to infer about gene functions. Two insertional mutagenesis approaches for gene disruption have been predominantly used in Arabidopsis: transferred DNA (T-DNA) [3] and transposon tagging [4].

Disadvantages of reverse genetics

Although these are powerful tools for providing novel mutants, they present some limitations, including the impossibility of studying the function of duplicated genes (multigene families), the difficulty to reach genome saturation, labor intensive, time consuming, and the multiple insertional nature of these approaches frequently leads to concomitant disruption of several genes and in some cases, unpredictable.

II. NEED FOR VIGS

These constraints can be circumvented by new PTGS tools, through which genes are silenced in a specific and efficient manner, using less intensive and quicker technologies compared to the conventional techniques [5]. The PTGS can be induced in plants by viral vectors harboring specific genes [6], through the virus-induced gene silencing (VIGS) system [7-8], by inverted repeat transgenes producing hairpin transcripts (hairpin RNAs, hpRNAs) [9], by asRNA technology [10] or by gene overexpression leading to co-suppression [11]. The VIGS system can be helpful to assess gene function, especially for genes that cause zygotic/embryo lethality when mutated and in species that are recalcitrant to genetic transformation.

III. DEVELOPMENT OF VIGS TECHNOLOGY

The term ‘‘VIGS’’ was first used by A. van Kammen to describe the phenomenon of recovery from virus infection [12]. However, the term has since been applied almost exclusively to the technique involving recombinant viruses to knock-down expression of endogenous genes [8]. The discovery of PTGS of endogenous genes by recombinant viruses carrying an identical sequence was made in 1995 [7]. Because it allows targeted downregulation of a particular gene through degradation of its transcripts, the potential of VIGS as a tool for the analysis of gene function was quickly recognized [8]. A DNA fragment with a minimum of 23 nucleotides bearing 100% identity to a targeted transgene appears to be required in order for silencing to occur [13]. However, a 23-nucleotide long sequence is often not sufficient to initiate silencing and longer identical sequences must be routinely used [14].

IV. MOLECULAR MECHANISM OF VIGS

It is likely that VIGS is caused by an RNA-mediated defense (RMD) mechanism in plants against viruses. According to this idea there is an as yet uncharacteriscd surveillance system in plants that can specifically recognise viral nucleic acids and give sequence specificity to RMD. Normally, with wild-type isolates of plant viruses, it is thought that this mechanism is activated as the virus begins to accumulate in the plant. Eventually, as a result of RMD virus accumulation slows down and eventually stops. In the situations when a genetically modified virus has similarity to a gene in the host plant. The RMD would target both the viral RNA and the corresponding endogenous mRNA. VIGS would result from the targeting of the endogenous mRNA.

V. METHODOLOGY OF VIGS

The most powerful aspect of VIGS as a tool for gene function studies and high-throughput functional genomics is the minimal amount of time and effort required to identify a loss-of-function phenotype for a gene of interest. The steps involved in VIGS are given in below (Figure 1). Initially, single gene sequences were subcloned individually into viral genomes and plants were inoculated by rubbing leaves with viral RNA produced by in vitro transcription reactions [7]. Although this approach is well-suited for studies looking at a limited number of genes, inoculating the plants in this manner is time-consuming and can yield variable results. Recent efforts to streamline the cloning process and subsequent inoculation of the virus have made it possible to go from gene sequence to phenotype in planta within 1 month, allowing a single lab to screen thousands of individual genes for a phenotype of interest in a high-throughput manner [15].

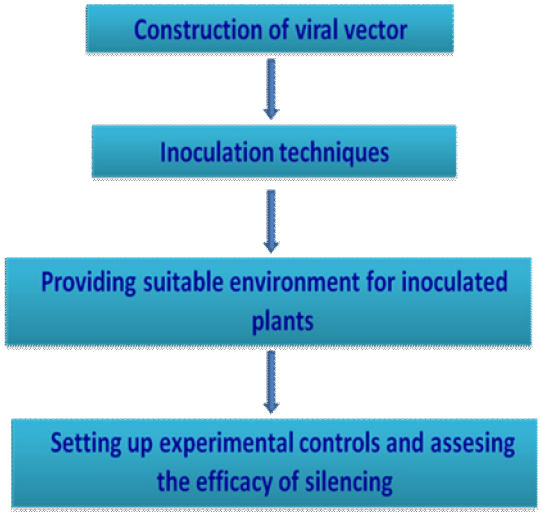

Figure 1. Overview of Virus Induced Gene Silencing

VI. VIRAL VECTORS USED TO STUDY GENE FUNCTIONS IN PLANTS

In early studies, VIGS was usually performed in the wild tobacco species Nicotiana benthamiana that is highly susceptible to virus infection and thus exhibits efficient gene silencing because of good infection. Tobacco mosaic virus (TMV) was the first RNA virus to be used as a silencing vector. Transcripts of recombinant TMV carrying a sequence from the phytoene desaturase (PDS) gene were produced in vitro and inoculated to N. benthamiana plants to successfully silence PDS [7]. A more recent VIGS vector is based on Potato virus X (PVX) [16]. Although this vector is more stable than the TMV-based vector, PVX has a more limited host range than TMV, with only three plant families having members that are susceptible to PVX infection compared with

nine families for TMV. Furthermore, both TMV and PVX-based vectors cause disease symptoms on inoculated plants, thus making interpretation of some subtle PTGS phenotypes difficult [17]. In addition, these viruses are excluded from the growing points or meristems of their hosts, which precludes effective silencing of genes in those tissues. The significance of this exclusion is still unclear as a VIGS vector based on the Tomato golden mosaic virus (TGMV) was used to successfully silence a meristem gene, proliferating cell nuclear antigen (PCNA) in N. benthamiana, although this virus is excluded from the meristem [18]. This TGMV-based vector had been previously used to silence a non-meristematic gene as well as a foreign transgene [19]. The limitations of host range and meristem exclusion were overcome with the development of VIGS vectors based on Tobacco rattle virus (TRV) [17]. 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 improved TRV VIGS vectors, pYL156 and pYL279, result in more efficient silencing of endogenous genes. These vectors differ from the earlier TRV vector by having a duplicated 35S promoter and a ribozyme at the C-terminus for more efficient production of viral RNA, as well as a number of amino acid changes in the viral sequence itself. Unlike the other VIGS vectors described so far, pYL156 and pYL279 are not limited to efficacy in N. benthamiana and they are being successfully used for silencing in tomato and other species [20]. TRV derived VIGS in S. rostratum was demonstrated by Meng et al., 2016. The number of plant species amenable to VIGS experiments is increasing with the development of new virus vectors. A two-component system (satellite virus-induced silencing system or SVISS) has been described that allows VIGS in tobacco, a species that is widely used in plant biology but which has proven recalcitrant to the use of other VIGS vectors [22]. This system consists of TMV (strain U2) and a satellite virus (STMV). Many vectors for use in VIGS had been developed since then.

A modified asymptomatic satellite DNA associated with Tomato yellow leaf curl China virus has also been used as a VIGS vector in N. benthamiana [23]. In the presence of the helper virus, the modified satellite, carrying marker genes gfp, su or pds caused silencing of the corresponding genes. To extend VIGS to the important tuber crop cassava (Manihot esculenta) a vector was developed from the DNA-containing geminivirus African cassava mosaic virus, carrying the gene encoding a subunit for the chloroplast enzyme magnesium chelatase (su), which is essential for chlorophyll synthesis, resulting in silencing of su in the leaves in 7–21 days post inoculation (dpi). Using the VIGS system described above, 70% reduction in the levels of the harmful secondary metabolite linamarine was achieved in [24]. For applying VIGS to legumes, the multicomponent RNA virus Pea early browning virus was appropriately modified [25] and several genes, including the marker genes gfp and pds and endogenous genes such as unifoliata, uni (homolog of the flo and lfy gene from Antirrhinum majus and Arabidopsis, respectively, involved in regulating compound leaf architecture) were successfully silenced in pea. Silencing of multiple genes was also successful but the silencing phenotype was milder than that resulting from individual silencing. For soybean, the multi-component RNA virus Bean pod mottle virus was developed as VIGS vector. To increase the stability of this vector, mutations in the predicted recombination sites were incorporated [26]. A VIGS vector has also been developed for silencing in barley, a monocotyledonous plant [27]. This barley stripe mosaic virus (BSMV) derived vector has been used to silence PDS. Indeed, the development of VIGS for functional genomics in monocots is significant because of the difficulty in applying other loss-of-function approaches requiring transformation to these species. A modified BSMV VIGS vector has been developed that has a greater efficiency of silencing as demonstrated by a more severe silencing phenotype. This new vector contains 40–60 bp direct inverted repeats that generate dsRNA upon transcription of the VIGS vector [28]. More recently, the monocot infecting Brome mosaic virus strain 'Tall-fescue' has been modified for VIGS and using which genes for PDS, actin and rubisco-activase have been silenced in the important model cereal rice [29]. One of the more interesting developments to improve the VIGS technology is the use of bipartite Cabbage leaf curl virus (CbLCV) to perform VIGS in Arabidopsis and requires particle bombardment for introducing it to the plant [30].

Besides CbLCV as a VIGS vector in this model species, only TRV has been reported to be effective for transient VIGS in Arabidopsis. This is the same TRV VIGS vector described by Ratcliff and his co workers in 2001 and used extensively for VIGS in N. benthamiana [17]. However, the protocol used for silencing in Arabidopsis requires that the TRV vector first be introduced into N. benthamiana to produce virions and then the virions are used secondarily to infect Arabidopsis [15]. A recently described alternative approach uses vacuum infiltration to introduce Agrobacterium into Arabidopsis plants [31]. Both of these procedures are time consuming and tedious, especially in large-scale functional studies. In an effort to generate a more useful set of tools for VIGS in this model dicotyledonous species, Burch-Smith have used TRV based VIGS vector [20] and optimized its delivery and effectiveness in Arabidopsis. They have demonstrated that VIGS can be used to examine the effects of silencing CUL1 (null mutant of which is embryo lethal) in adult tissues. Using the same system, silencing of two disease resistance genes,

rpm1 and rps2 along with gfp, led to loss of resistance against the pathogen Pseudomonas syringae and this could be co-localized to gfp-silenced regions of leaves [20]. The above silencing phenotypes correlated well with reduction of their corresponding transcripts. With these modifications, previously published protocols for VIGS in N. benthamiana or tomato can be used for silencing in Arabidopsis [15]. This provides an avenue for large-scale functional genomic screens in Arabidopsis, as has been performed in N. benthamiana. Thus, TRV-based VIGS holds promise as a powerful tool for genetic analysis in this important model plant.

VII. CONCLUSION

VIGS shows much promise as a tool for gene function studies and for high-throughput functional genomics in plants and it has already begun to fulfill some of this promise. Also, there is still a great potential for this approach that remains to be tapped. The utility of the existing vectors, especially TRV-based ones, would be greatly improved with the increased availability of gene sequences for plants in which these vectors are functional. Using VIGS to target a specific gene requires sequence information. Excitingly, there has been progress towards the sequencing of plant genome by an international collaboration. Such a development will address some of the concerns about the specificity of silencing in crops and make it much easier to identify targets and design primers to carry out VIGS. Identifying genes that regulate interesting phenotypes through VIGS could also be achieved by large-scale screens. Thus, as VIGS becomes adapted to more plant species and researchers become more familiar with the technique, we expect to see it become a common and widespread tool for gene function studies and functional genomics in plant biology.

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