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Recent Advances on Virus Induced Gene Silencing (VIGS): Plant Functional Genomics

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Introduction

In recent decades, availability of ample information of various plant genomes has reached the hand of plant researchers through genome sequencing and expressed sequence tag (EST) analysis that eventually facilitate the functional study of wide range of genes. The previous work on plant functional genomics were exclusively based on forward genetics; that is, identification of a mutant and subsequent cloning of the mutated gene to investigate the wild-type phenotype of target gene. With the advent of the reverse genetics an important alternative approach was to directly alter expression of the gene sequence of interest to be analysed and subsequently identify the mutant phenotype produced after changing their expression. Virus-induced gene silencing (VIGS) has been used extensively with great potential in plant reverse genetics for the past few years. It is the simplicity, quick and cost effectiveness of the method that makes VIGS instrument as an attractive alternative post transcriptional gene silencing (PTGS) method for studying gene function and high-throughput functional genomics. It is used to identify a loss-of-function phenotype of a desire gene involved in basic cellular functions, metabolic pathways, development biology, plant-microbe interaction, and abiotic stress. A. van Kammen first used the term "VIGS" to describe the phenomenon of recovery from virus infection [1].

Mechanism

A plant virus infects a host cell and activates an RNA-based defence that target the viral genome. Virus-induced gene silencing—VIGS—is a virus vector technology that exploits this RNA-mediated defence mechanism. After the infection the replication of viral RNA proceeds and produces a dsRNA replication intermediate which in turn produce siRNA in the infected cell that correspond to parts of the viral vector genome, including any non-viral insert. The siRNA in the infected cell guide an RNase complex by base pairing and specifically knock out the expression of single-stranded (ss) target RNA recognized by the dsRNAs. Thus, if the insert is from a host gene, the siRNA/RNase Complex would target the RNase complex to the respective host mRNA and the symptoms would appear in the infected plant as the loss of the function of the target protein (Figure 1).

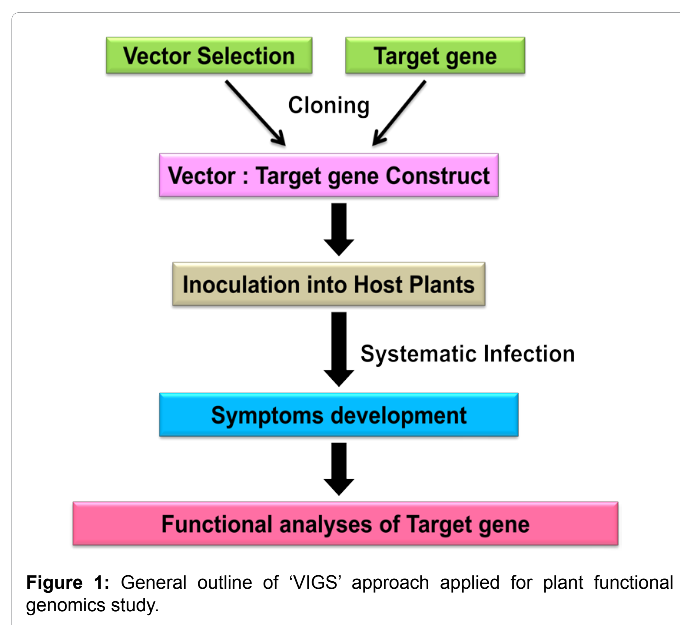

Figure 1: General outline of 'VIGS' approach applied for plant functional genomics study.

Recent Advances on VIGS in Plant Functional Study

The first RNA virus used as a silencing vectors is *Tobacco mosaic virus* (TMV) [2]. Earlier 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 [2].

Pepper huasteco yellow veins virus (PHYVV)-derived vector were used to postulate the involvement of three genes *Comt* (encoding a caffeic acid O-methyltransferase), *pAmt* (a putative aminotransferase), and *Kas* (a β-keto-acyl-[acyl carrier-protein] synthase) genesin the biosynthesis of capsaicinoids which are responsible for the pungent taste of chilli pepper fruits of Capsicum species and expressed differentially n placenta tissue [3]. They have successfully produced non-pungent chilli peppers at high efficiency with these VIGS approach. Silencing efficiency and the applicability of silenced plants for herbivore feeding assays were investigated infecting *Tobacco rattle virus* (TRV) vectors using leucine amino peptidase (LAP), herbivore-induced protein. Empty-vector controls reduced plant growth while control vectors consisting of a piece of noncoding sequence did not affect the plant growth. They have found that silencing of LAP profoundly increased the larval mass of *Manduca sexta* which fed on silenced plants demonstrating its involvement in defensive function against herbivores [4]. *Agrobacterium tumefaciens* mediated transfer of *CvPDS* gene (*Phytoene desaturase*) and *CvFLO*gene (Floricaula) into *Cysticapnos vesicaria* (Papaveraceae, basal eudicots) through *Tobacco rattle virus* (TRV)-based vectors have resulted in strong photobleaching of green parts and reduction of endogenous *CvPDS* transcript levels and affected floral phyllotaxis, symmetry and floral organ identities respectively [5]. Glutathione S-transferase (GST) was known to be up-regulated during the infection of fungal pathogen

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O. neolycopersici in resistant tomato plants (*Solanum habrochiates* G1.1560) that resulted in the accumulation of H_2O_2 and Hypersensitive reaction (HR) and retarded the growth of the pathogen. VIGS silencing experiments was performed to knock-down expression of the putative GST gene (ShGST) in resistant tomato plants (*Solanum habrochiates* G1.1560) carrying the Ol-1 gene and showed that the TRV-ShGST-silenced plants decreased HR and H_2O_2 accumulation which might be insufficient for the tomato plant to prevent sporulation of *O. neolycopersici* and behaved as susceptible one after inoculation with *O. Neolycopersici* [6]. Infection with an *apple latent spherical virus* (ALSV) vector containing a fragment of soybean isoflavone synthase 2 (soyIFS2) gene reduced the levels of both soyIFS2- and soyIFS1- mRNAs and subsequently the isoflavone content in the cotyledons of mature seeds of infected soybean plants [7]**.** *Rice tungro bacilliform virus* (RTBV) has been developed as a rice-infecting virus containing DNA as thegenetic material. *Agrobacterium* (agroinoculation) mediated transfection accumulated a full-length RTBV DNA cloned as apartial dimer in a binary plasmid and produced detectable RTBV coat proteins within 2 weeks of infection [8]. *Cucumber mosaic virus vector* (CMV-A1) was used to analyseVIGS containing partial sequence of AINTEGUMENTA gene of *Antirrhinum majus* (Am-ANT) that inhibited the expression level of Am-ANT mRNA when infected by the vector. The phenotypic expression has been observed for the A1:ANT-infected *Antirrhinum* plants which corroborated the smaller floral organs and leaves although cell sizes remained unaltered in flowers and larger in leaves[9]. *Apple latent spherical virus* (ALSV) vectors were constructed carrying sequence of endogenous genes from apple [ribulose-1, 5- bisphosphate carboxylase small subunit (*rbcS*), alpha subunit of chloroplast chaperonin (CPN60a), elongation factor 1 alpha (EF-1a), or actinby a particle bombardment which lead to knock-down phenotypes of each gene on the true leaves of 2~3 weeks seedlings after inoculation. In addition to that TERMINAL FLOWER 1 gene of apple (MdTFL1) was studied using this vector construct to show precocious flowering which is as expected as a knock-down phenotype of the silencing of MdTFL1 gene [10]. *Barley Stripe Mosaic Virus* (BSMV)-VIGS construct has been used to investigate the influential role of two LEA genes (HVA1 and Dhn6) during drought tolerance in *Hordeum vulgare* [11]. HMW-GS 1Bx14, a gene encoding for High-molecular-weight glutenin subunit 1Bx14, has been proved as one of major components involved in the formation of glutenin macro polymers in wheat grains based on BSMV vector [12].Three genes of *Triticum aestivum, Era1* (enhanced response to abscisic acid), *Cyp707a* (ABA 8'-hydroxylase), and *Sal1* (inositol polyphosphate 1-phosphatase) were cloned in the viral vector *Barley Stripe Mosaic Virus* (BSMV) followed by rub inoculation of BSMV viral RNA transcripts into wheat indicated that *Era1* and *Sal1* play important roles in conferring drought tolerance whereas *Cyp707a*-silenced plants showed no improvement under limited water condition [13]. Serine hydroxyl methyltransferase (SHMT) gene in *Glycine max* introduced in *Bean Pod Mottle Virus* (BPMV) conferred resistance to foliar pathogens, soybean cyst nematode (SCN) [14]. Five transcription factors involved in various aspects of flower morphogenesis in the orchid *Phalaenopsis equestris* namely *PeMADS1* (*AGAMOUS*-like MADS-box gene), *PeMADS7* (D-class MADS-box gene), *PeHB, PebHLH* and *PeZIP* were inserted into orchid viruses, *CymMV* to explore their roles during flower development. Silencing of PeMADS1 and PebHLH gene lead to reduced flower size together with a petaloid column containing petal-like epidermal cells and alterations of epidermal cell arrangement in lip lateral lobes, respectively, whereas silenced PeMADS7, PeHB, and PeZIP genes alone resulted in abortion of the first three fully developed flower buds of an inflorescence [15].

Considering all the evidences it can be concluded that VIGS can stronglybe used as a tool for gene function studies but also can be used for high-throughput functional genomics in plants. VIGS vectors have provided the extreme trust worthy and effectual results to the selective host range. Most of them are functional only in *N. benthamiana* and other members of the Solanaceae family like tomato. In future, it is required to develop wider host ranges of the vector that can successfully influence the effective VIGS in *Arabidopsis* and rice in particular that will offer future endeavour to the present plant research community.

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