Virus Induced Gene Silencing In Plant Disease Management

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Abstract

Virus induced gene silencing (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.

Keywords –Virus induced gene silencing,Agro-infiltration, Agrobacterium

Introduction

Gene silencing is described as epigenetic processes of gene regulation. Gene silencing is a technique used to turn down or switch off the activity of genes by a mechanism other than genetic modification. That is, a gene which would be expressed (turned on) under normal circumstances is switched off by machinery in the cell (Robertson, 2004). More specifically. gene silencing (GS) is defined as a molecular process involved in the down regulation of specific genes, the mechanisms of Gene silencing that suppress gene activity in plants has extended that control of gene expression. Currently, there are several routes of GS identified in plants, such as: transcriptional gene silencing and post transcriptional (PTGS or RNAi) gene silencing (Voinnet, 2005) microRNA silencing and virus induced gene silencing.

Different methods of delivering the VIGS vectors into plants:

Woody plants have been successfully inoculated with engineered viruses using particle bombardment (biolistic) of apple cotyledons with viral RNAs, of the cDNA behind an appropriate promoter, by agro-inoculation of stem incisions or leaf infiltration.

i. DNA rubbing:RNA transcripts and plasmids are applied by mechanical means by rubbing suspensions on the plant leaf together with a fine abrasive.

ii. Biolistic:Virus vectors of difficult-to-inoculate hosts can be coated onto gold particles and inoculated using a biolistic apparatus (i.e., gene gun).

iii. Agro-inoculation/ agro-infiltration:The injection of Agrobacterium carrying similar DNA constructs into the intracellular spaces of leaves for triggering RNA silencing is known as agro-inoculation or agro-infiltration. The simple mechanism for inducing RNAi is like the strategy for transient expression of T-DNA vectors after delivery by Agrobacterium tumefaciens. The transient plants were then transformed with A. tumefaciens genes harbouring the gene of interest for inducing RNAi (Burch-Smith et al., 2004).

iv. Leaf Injection:Leaf injection (syringe infiltration) is the most common agroinfiltration method used for leaf or floral development related gene studies in dicots with high silencing efficiency silenced the NbPDS gene in tobacco by infecting N. benthamiana leaves with a 2-ml needleless syringe carrying TRV Agrobacterium cultures and later novel method was first proposed for VIGS (Schultinket al., 2019).

Mechanism of VIGS in plants

VIGS often relies on only the viral genome being delivered to the plant cell, e.g., via A. tumefaciens - mediated transformation, with the genome integrated into A. tumefaciens' T-DNA requiring host-cell-dependent transcription to produce the first viral particles. Following agroinfiltration of an expression cassette containing a cDNA copy of the genome, a single-stranded mRNA resembling the virus is transcribed by the

host RNA polymerase and exported to the cytoplasm. The first sequence on the mRNA is then translated to produce the viral replicase (an RdRP). Replication of similar mRNA molecules containing a 30s -virus-specific recognition sequence is initiated and the viral vector moves into new cells. RNA viruses with multiple genome components, such as TRV, can be cloned and co-inoculated with high efficiency. Most RNA virus–derived vectors are insertion vectors and contain a duplicated sub genomic promoter preceding the insertion site (Rossner et al., 2022).

Factors affecting VIGS efficiency:

i. Viral Vector Selection:To simplify VIGS protocols, several different approaches to subcloning and virus inoculation have been developed.

a. Agrobacterium Strain: The optimal A. tumefaciens strain used for VIGS varies with different plants and affects the gene silencing efficiency. Studies have shown that Agrobacterium strain GV2260 works best in N. benthamiana, while strain GV3101 could also be used.

b. Inoculum Concentration: As the agro-inoculation methods and characteristics of diverse infected plants are different, the concentration of the infection solution strongly affects the gene silencing efficiency of VIGS experiments.

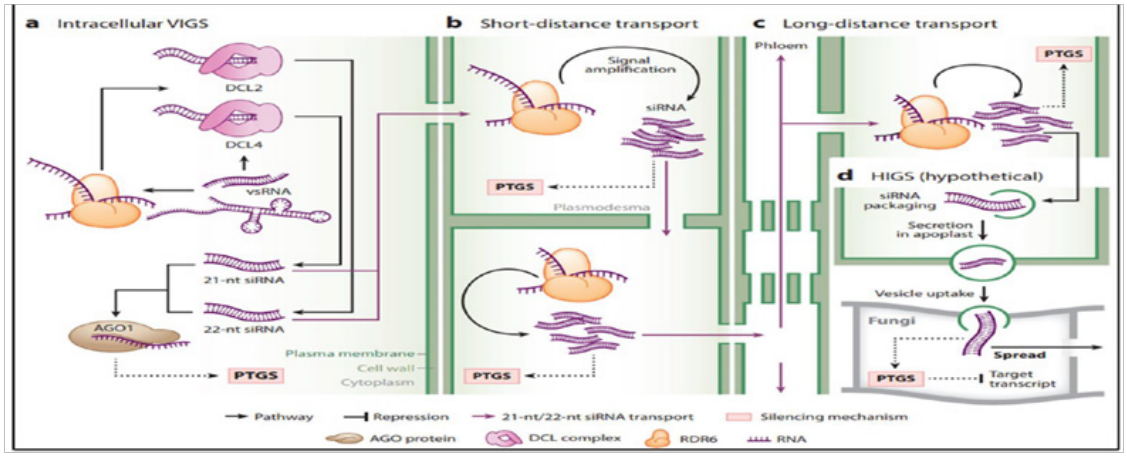

Fig 1- Systemic and interspecific spread of siRNA signals by VIGS vector

Types of VIGS:

The available vector systems can be divided based on the molecular nature of the vector.

(a) ssRNA viruses such as TRV, used for a wide variety of dicots, or the barley striped mosaic virus (BSMV), applied to many Poaceae crops.

(b) ssDNA viruses such as the tomato mottle virus (ToMoV) or ACMV, both with a more limited host range (e.g., only N. benthamianaand Manihot cassava for ACMV).

Many viruses distribute their genomes in two (e.g., TRV and ToMoV) or three fragments (e.g., BSMV) of which only one will be modified by inserting part of the target gene transcript sequence for VIGS (Cui et al., 2018).

RNA-virus-induced gene silencing (RVIGS):

The majority of the VIGS vectors used for gene silencing are RNA-based viruses because RNA viruses have been shown to induce silencing in a variety of host plants, including Capsicum frutescens (chili pepper), Capsicum (bell pepper), Arabidopsis thaliana (thale cress), and several other monocotyledon plants, such as Oryza sativa (rice), Hordeum vulgare (barley), and Zea mays (maize), to eliminate endogenous transcripts (Kant et al., 2021).

Conclusion:

Virus-induced gene silencing (VIGS) is an RNA-mediated reverse genetics technology that has evolved into an indispensable approach for analyzing the function of genes.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 fulfil some of this promise. However, there is still a great potential for this approach that remains to be tapped. VIGS are more widely used for large-scale screens to identify interesting phenotypes. Thus, VIGS has become a common and widespread tool for gene function studies and functional genomics in plant biology.

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