Scientific Research Publishing

American Journal of Plant Sciences, 2019, 10, 1649-1661
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Advances in the Application of Virus-Induced Gene Silencing in Plants

Ying Zhang[1], Huize Chen[1,2*], Rong Han[1,2*]

[1]Higher Education Key Laboratory of Plant Molecular and Environment Stress Response, Shanxi Normal University, Linfen, China
[2]School of Life Science, Shanxi Normal University, Linfen, China
Email: zysxnu@hotmail.com, *chenhuize@hotmail.com, *hhwrsl@163.com

How to cite this paper: Zhang, Y., Chen, H.Z. and Han, R. (2019) Advances in the Application of Virus-Induced Gene Silencing in Plants. *American Journal of Plant Sciences*, **10**, 1649-1661.
https://doi.org/10.4236/ajps.2019.109117

Received: April 16, 2019
Accepted: September 26, 2019
Published: September 29, 2019

Abstract

VIGS (Virus-induced gene silencing) can effectively silence target genes at the RNA level to investigate their functions. The virus vectors used for silencing are divided into three categories, DNA virus, RNA virus, and satellite virus, which can be used to silence the target genes in different species based on existing research. The genes used as markers for silencing system identification include PDS and PCNA. VIGS can be applied to some plants, in particular cash crops and fruit trees, to study their disease-resistance genes and genes related to growth and development to provide a basis for improving crop characteristics.

Keywords

VIGS, Application, VIGS Vector, Marker Genes

1. Introduction

Traditional methods to study plant gene function include transgenic technology, gene knockout, the gene induced overexpression, and RNAi technology. These research methods all have certain limitations, such as long research cycle, the need for genetic transformation and low conversion efficiency, which limit the rapid and efficient application of these methods in the study of plant gene function [1] [2]. However, virus-induced gene silencing (VIGS) is a rapid and efficient tool for plant functional genomics research because it permits knockdown of genes-of-interest and observation of elicited phenotypes within 3 to 4 weeks [3]. VIGS is a post-transcriptional gene silencing technique [4] and has been used as a method to study resistance genes and functional genes of many cash crops.

The molecular mechanism of VIGS is shown in Figure 1; the fragment of the target gene is inserted into the viral vector, the constructed viral vector is introduced into *Agrobacterium*, and then the plant is infected with *Agrobacterium*. After the virus enters the plant cells, it will through replication and transcription using various raw materials in the plant cells to form a double-stranded dsRNA. The double-stranded dsRNA in the cells will then be recognized by Dicer analogs of specific RNAase III family end onucleotides and small interfering (si)RNA of 19 - 24 nt will be cut. siRNA binds to RNase in plants as a single strand to form an RNA-induced silencing complex. In the cytoplasm, the mRNA of the target gene will bind to the complex, which leads to the degradation of the mRNA of the target gene. The infected plant would have the same phenotype as a deletion mutation of the target gene, and thus the function of the target gene could be inferred based on the change in the trait [5] [6] [7] [8].

In different RNA-induced silencing systems [9], VIGS can be used for transient transformation and has advantages, such as simple and routine use in a single generation without requiring a long time to screen many transgenic plants, over methods that require permanent modification [1] [2] [10]. Therefore, VIGS is considered to be a powerful reverse genetics technique for studying the function of plant genes [11] [12]. It has been successfully developed and applied in several dicotyledonous plants and some monocotyledonous plants [13] and has been widely used to identify genes related to abiotic stress tolerance [14] [15].

2. Types of Virus Vectors and Marker Genes

Viruses used to carry the target gene fragment are divided into DNA viruses, RNA viruses, and satellite viruses (Table 1). The first VIGS vectors, derived from tobacco Mosaic virus (TMV) [16], potato virus (PVX) [17] [18], and tobacco bell virus

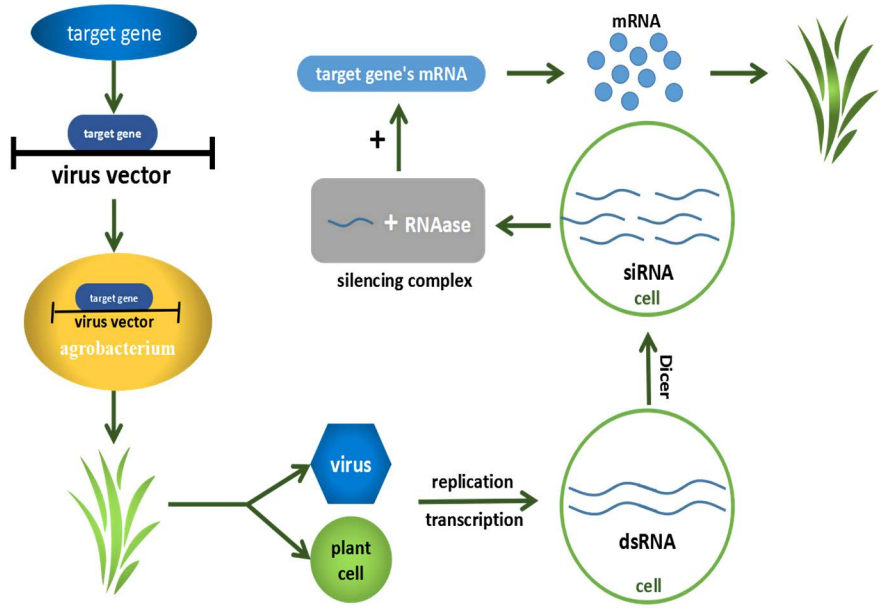

Figure 1. VIGS molecular mechanism of action.

Table 1. Types of virus vector [62] [63].

Virus vector	Virus genus	Silenced hosts
RNA virus		
Barley stripe mosaic virus	*Hordeivirus*	Barley, Wheat
Bean pod mottle virus	*Comovirus*	*G. max*
Brome mosaic virus	*Bromovirus*	Barley, rice, maize
Pea early browing virus	*Tobravirus*	*P. sativum*
Poplar mosaic virus	*Carlavirus*	*N. benthamiana*
Potato virus X	*Potexvirus*	*N. benthamiana*, *S. tuberosum*
Tabacco mosaic virus	*Tobamovirus*	*N. benthamiana*, *N. tabacum L.*
Tobacco rattle virus	*Tobravirus*	Arabidopsis, *N. Benthamiana*, *Tobacco*
DNA virus		
African cassava mosaic virus	*Begomovirus*	*N. Benthamiana*
Cabbage leaf curl virus	*Begomovirus*	Arabidopsis
Satellite virus		
Satellite tobacco mosaic virus		*N. tabacum L*

(TRV) [19] [20], were originally used for silencing the genes of *Nicotiana benthamiana* and *Solanum lycopersicum.*

2.1. RNA Virus Vectors

BSMV (*Barley striped mosaic virus*) is an orthomolecular RNA single-stranded virus, consisting of three RNA chains: α, β, and γ [21]. RNA α of BSMV ND18 encodes RNA polymerase (RdRp)-dependent methyltransferase/helicase subunits, and RNA β encodes special envelope proteins (CP), and three major proteins essential for virus movement between cells (TGB1, TGB2, and TGB3). RNA γ encodes RdRp polymerase subunit (GDD) and γb proteins, which are involved in viral pathogenesis, long-distance movement and suppression of host RNA silencing defenses. When wheat leaves are infected, symptoms are irregular stripes of dark green and light green. In addition, it appears as yellow and white stripes in early stage new leaves, along with decreased plant height, reduced pistil fertility, and dry seeds [3].

BMV (*Brome mosaic virus*) is a tripartite, single-stranded, positive-sense RNA virus [22]. BMV genomic RNA1 and RNA2 encode 1a and 2a proteins, respectively; both are required for virus replication [23] [24] [25]. BMV genomic RNA3 is dicistronic, encoding a 3a protein required for virus cell-to-cell movement in the plant and a capsid protein (CP) necessary for virion formation and virus movement in the plant [26] [27] [28] [29]. The CP is translated from a subgenomic RNA (RNA4). For both reported BMV vectors, foreign gene fragments are inserted into genomic RNA3 [30] [31] [32] [33].

TRV (*Tobacco rattle virus*) is a soil-borne virus belonging to the RNA virus

family. The sedimentation coefficient of TRV virus is 296 - 3065 and 115 - 2455 S, and the virion length is 180 - 215 and 46 - 114 nm. Virions are straight rods; there are two kinds of virions: long virions (190 - 210 nm × 25 nm) and short virions (40 - 80 nm × 20 - 25 nm), and the ratio of long virions to short virions is 1:2 [34]. The virus genome contains two RNA strands, RNA1 and RNA2. RNA1 encodes RNA polymerase genes, and cysteine-rich proteins, and RNA2 encodes capsid proteins and enzyme cleavage sites of cloned target genes; the virus particles are extremely stable. Two chains of RNA1 and RNA2 constitute binary vectors, so TRV-induced gene silencing requires simultaneous action of RNA1 and RNA2 [19] [35]. RNA 1 encodes sufficient protein for replication and movement within host plants. RNA 2 encodes proteins that allow virions to form and spread between plants, mediated by nematodes [36].

2.2. DNA Virus Vectors

African cassava mosaic virus (ACMV) is a single-stranded (ss)DNA virus, belong to the Begomovirus genus [37]. Geminiviruses are small, circular DNA viruses that can be inoculated into plants using *Agrobacterium* or microprojectile bombardment of plasmid DNA [38]. The genome of ACMV is composed of DNA-A and DNA-B, which are approximately 2.7 kb; both are required for systemic infection of plants. It is necessary for replication, and transcriptional regulation of viral gene expression to DNA-A and DNA-B are almost identical in homologous areas containing cis-acting elements. The two-part ACMV genome encodes eight proteins responsible for the viral life cycle and movement among host plants. The multifunctional replication initiator protein (Rep) is essential for the initiation of rolling circle replication (RCR) of both DNA A and DNA B. Rep can also act as a transcription inhibitor, causing hypersensitivity and virus resistance in plants. ACMV infection can induce antiviral RNA silencing defenses, affect siRNA production, disturb microRNA biosynthesis, and cause abnormal developmental phenotypes in plants [37].

2.3. Satellite Tobacco Mosaic Virus

STMV (Satellite tobacco mosaic virus) is a natural two-component system that isolates virus replication and movement from silencing-inducible components. This configuration can enhance the stability of the system. Owing to the small genome of STMV (single tRNA of 800 - 1300 bases), satellite virus RNA has a high replication efficiency and accumulates to high numbers in infected plants. In addition, because the genome is small, it is easy to obtain the infectious clones needed for vector construction. Because the satellite virus RNA does not have a cap and does not polyadenylate in vivo, there is no need to protect the satellite virus transcript through laborious and expensive in vitro end modification until it has been inoculated. Satellite viruses can alleviate symptoms caused by helper viruses (Roossinck *et al.* 1991) and allow sensitive screening for phenotypic changes in knockout plants [39].

3. Marker Genes for VIGS

VIGS will lead to the occurrence of genotypic phenotypes in plants after the silencing of endogenous target genes, and genes that determine the success of the silencing system are called marker genes (Table 2).

PDS (*phytoene desaturase*) genes serve as a convenient visual indicator for VIGS [5]. Plants that successfully silenced PDS include barley, wheat, soybean, rice, tobacco, and *Arabidopsis thaliana* [40] [41] [42] [43]. The silenced plants showed photo-bleaching. After the silencing system was stable, the phenotype of the gene defect could be observed more readily in the plants (Figure 2).

PCNA (*proliferating cell nuclear antigen*), a highly conserved processing factor for DNA polymerase *δ*, is required for DNA replication and repair and is highly expressed in dividing cells. When PCNA was silenced, young leaves continued to expand, forming cabbage like clusters at the apical meristems. Leaves were often misshapen with truncated basipetal growth and little or no petiole development (Figure 3).

Table 2. Types of marker gene [63].

Gene		Function	Silence phenotype
PDS	*phytonene desaturase*	Carotenoid synthesis related enzymes	Leaf blade bleaching
pcna	*Proliferating cell Nuclearantigen*	Factor of DNA polymerase *δ*	Meristems stop growing
ChlH	*magnesium chelatase*	Enzymes involved in chlorophyll synthesis pathways	Leaves bright yellow
ChsA	*chalcone synthasa*	Enzyme of anthocyanin synthesis pathway	A part or whole of a design or color that turns white
su	*Sulfur gene*	Magnesium ion chelase in chlorophyll synthesis pathway	superior leaf
rpIIa	*RNA polymeraseII-a*	RNA synthesis	Leaves twisted and whitened
gfp	*green fiuorescent protein*	Fluorescence occurs under ultraviolet excitation	The leaves appear red under ultraviolet light
luc	*luciferase*	Fluorescence occurs under ultraviolet excitation	There was no fluorescence under ultraviolet light

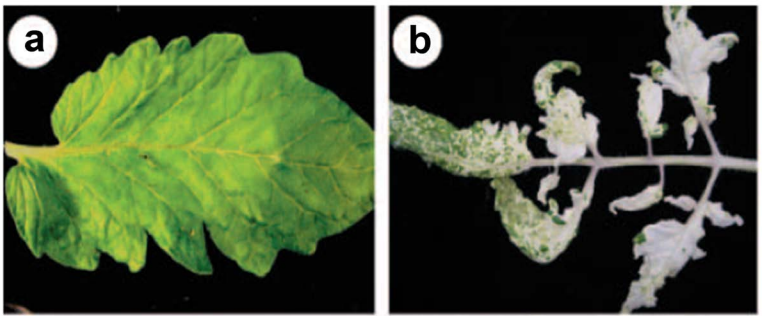

Figure 2. PDS silencing phenotype. (a) Tomato leaf infected with TRV virus only. (b) PDS gene silenced in tomato using TRV construct. Photobleached tissue indicates regions of silencing [1].

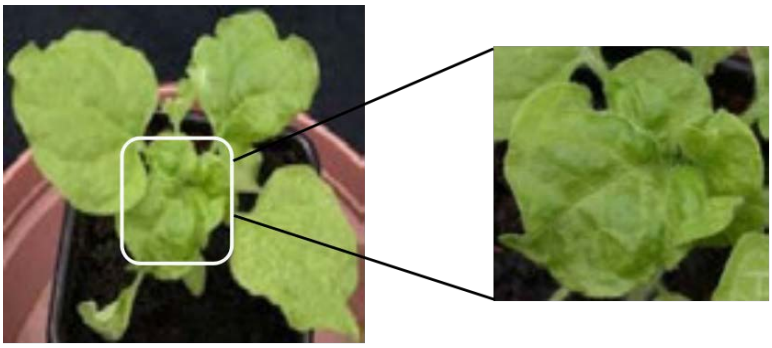

Figure 3. VIGS of NbPCNA caused abnormal leaf development and growth retardation [37].

4. Application of VIGS in Plants

The phenotype of a gene defect after utilization of VIGS technology is readily apparent, so it is more widely used in research of crop disease-resistance genes. After silencing the relevant resistance genes using VIGS technology, inoculation of bacteria will occur and the associated stress will be imposed. The phenotype of the plant can clearly be observed. In addition, some environmental stresses and the emergence of crop diseases may lead to the decline of cash crop yield, so functional research on related resistance genes is very important for improving the characteristics of affected crops.

4.1. Studies on the Function of Disease-Resistance Genes

In the study of resistance genes related to leaf rust, Scofield *et al.* silenced wheat varieties Lr21, RAR1, SGT1 and HSP90 by BSMV-VIGS and found that these genes played an important role in the lr21-mediated resistance pathway [44]. In the study of resistance to powdery mildew, Wang *et al.* used BSMV-VIGS to silence Hv-LRR in *Haynaldia villosa* and analyzed its role in resistance to powdery mildew [45]. Várallyay *et al.* used the BSMV-VIGS system to construct the BSMV: PDS: Mlo vector has realized the labeled gene with the target gene silence system, promoted the VIGS system more widely used [46]. MLO, a transmembrane protein, plays a negative regulatory role in inhibiting plant defenses in uninfected tissues. It protects against cell death and the response to biotic and abiotic stress. In this study, wheat plants showed broad-spectrum powdery mildew resistance at the individual level after the lack of expression of this functional protein. Delventhal *et al.* used BSMV-VIGS system-silenced MLO in barley. High resistance to powdery mildew was also found [47]. Further, the resistance gene of barley powdery mildew was studied. Hein *et al.* used VIGS to confirm the requirement for Sgt1, Rar1, and Hsp90 genes in the Mla13-mediated resistance response to powdery mildew in barley, as previously observed in other R gene-mediated responses in dicot species including *Arabidopsis* and *Nicotiana* [48].

4.2. Investigation of Abiotic Stress Resistance Genes

In the study of abiotic stress resistance genes, Zhang *et al.* (2018) used VIGS and

iTAQ and found that TaGRP2, CDCP, and Wcor410c potentially play vital roles in conferring osmotic-cold tolerance in bread wheat. Tavakol (2017) silenced AetDreb2, a member of the ERF family, which accelerates plant wilting and reduces RWC under dehydration conditions. Further, silencing of the ERF109 gene confirmed its influence on tolerance to salt stress in *Arabidopsis* [49]. In addition, two new members of the ERF family in pepper (CaPTI1) and *P. somniferum* (PsAP2) affected the regulation of defense-related genes and caused a significant reduction in the AOX1 level through VIGS [50] [51]. Zhang *et al.* (2018) used TRV-VIGS to elucidate the effect of ghb1-1 on salt resistance of cotton seed. BSMV-VIGS was utilized to silence the SAMS, SAMDC and γ-ECS genes, and their relationship with drought resistance was analyzed. Silenced plants were compared to normal plants after drought stress. Silenced plants were more susceptible to the effects of drought stress, and the SAMS, SAMDC and γ-ECS genes were associated with drought resistance and water saving functions. These results showed that the S-adenosine methionine metabolism pathway in wheat drought resistance and water saving has potential value [52]. Kuzuoglu-Ozturk *et al.* (2012) used BSMV-VIGS to confirm that Atg8-silenced plants exposed to osmotic stress had decreased Atg8 expression levels in comparison to controls. Thus, Atg8 is a positive regulator in osmotic and drought stress responses.

4.3. Study of Genes Related to Growth and Development

The function of TaRSR1 in starch synthesis of wheat grains was explored using VIGS by Liu *et al.* (2016) In addition, in research related to the Wheat root length gene of COI1 [53], VIGS of the P23k gene led to abnormal leaf development, asymmetric orientation of main veins, and cracked leaf edges caused by mechanical weakness. Further, the involvement of P23k in the synthesis of cell wall polysaccharides and contribution to secondary wall formation in barley leaves were confirmed [54]. In plants, phospholipase D (PLD) is a key component of signal transduction in diverse pathways, including programmed cell death, senescence, and responses to biotic and environmental stresses. This research using BSMV-BASED VIGS found that the PLD gene was related to environmental stress responses [55]. Further, Ding *et al.* (2006) used VIGS technology to silence actin protein in rice. In addition, the function of TaRSR1 in starch synthesis in bread wheat used VIGS [56]. Bread wheat (*Triticum aestivum* L.), one of the major staple crops in the human diet, is an essential component of global food security [57]. This research can help improve crop varieties. In the study of tomato fruits, by silencing LeACS2, LeCTR1, and LeEILs, their role in fruit development was discovered [20]. This research also promoted studies related to fruit development using VIGS.

5. Future Prospects

VIGS is a reverse genetic method for studying the function of plant genes. It uti-

lizes RNA-mediated post-transcriptional gene silencing and acts as an antiviral defense system in plants. VIGS, as a tool, has the advantages of being simple to perform, highly efficient, and independent of genetic transformation in comparison with traditional technology and is suitable for analysis of large-scale gene function and lethal phenotype gene analysis, and so on [58]. However, this technique still has many limitations, such as limited inoculation methods between different plants and difficulty in observing characteristics of some genes after deletion. Still, the rapid and efficient characteristics of this technique cannot be ignored. In recent years, geminivirus vectors that can infect many plant species, including wheat, have been developed for exogenous protein expression. For other viral systems, inserted genes have limited cargo capacity in the proto-viral system. However, the insertion of larger foreign genes is possible, which involves the suppression of important genes encoding motor proteins and shell proteins, thereby eliminating intercellular movement or plant-to-plant transmission and inhibiting virus replication [59]. In addition, after the deletion of genes related to nutrient growth and resistance to diseases and insect pests and environmental stress, plants can show clearly observable characteristics, which also is favorable for the study of the functions of these genes.

Recently, the expression of GFP and iLOV and co-expression with the target gene has been studied. In addition, there have been studies on the expression effects of GFP with different fragment sizes in hosts carrying the VIGS vector [7] [21]. Cheuk and Houde (2017) changed the components of BSMV to confirm that different amounts of components had different cargo capacities [60]. This research can allow changes in virus vectors, so that they can carry at least two gene fragments, which would permit more gene functions to be determined. Thus, when we build vectors, we can put a target gene and a marker gene in the vector or two genes that can produce phenotypes, and then silence and study them. In this way, we can use VIGS technology to study more gene functions.

For inoculation methods, the characteristics of plants studied at different growth stages are also limited owing to the existence of virus transmission time in many current methods. Zhang *et al.* (2017) used vacuum and co-cultivation agroinfiltration of germinated seeds, which can greatly advance the growth stage of silenced plants, and is useful in studying the characteristics of plants at the seedling stage [61].

VIGS technology plays a major role in improving the characteristics of some crops and fruit trees, which promotes the development of cash crops.

Acknowledgements

This work was supported by the Postgraduate Innovative Project of Shanxi Province (2018SY061) and Postgraduate Innovative Project of Shanxi Normal University (2017SCX045).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Burch-Smith, T.M., Anderson, J.C., Martin, G.B. and Dinesh-Kumar, S.P. (2004) Applications and Advantages of Virus-Induced Gene Silencing for Gene Function Studies in Plants. *Plant Journal*, **39**, 734-746. https://doi.org/10.1111/j.1365-313X.2004.02158.x

[2] Lu, R., Martin-Hernandez, A.M., Peart, J.R., Malcuit, I. and Baulcombe, D.C. (2003) Virus-Induced Gene Silencing in Plants. *Methods*, **30**, 296-303. https://doi.org/10.1016/S1046-2023(03)00037-9

[3] Yuan, C., Li, C., Yan, L., Jackson, A.O., Liu, Z., Han, C., *et al.* (2011) A High Throughput Barley Stripe *Mosaic Virus* Vector for Virus Induced Gene Silencing in Monocots and Dicots. *PLoS ONE*, **6**, e26468. https://doi.org/10.1371/journal.pone.0026468

[4] Yu, J., Yang, X.D., Wang, Q., Gao, L.W., Yang, Y., Xiao, D., *et al.* (2018) Efficient Virus-Induced Gene Silencing in *Brassica rapa* Using a Turnip Yellow Mosaic Virus Vector. *Biologia Plantarum*, **62**, 826-834. https://doi.org/10.1007/s10535-018-0803-6

[5] Ramanna, H., Ding, X.S. and Nelson, R.S. (2013) Rationale for Developing New Virus Vectors to Analyze Gene Function in Grasses through Virus-Induced Gene Silencing. In: Becker, A., Ed., *Virus-Induced Gene Silencing. Methods in Molecular Biology* (*Methods and Protocols*), Humana Press, Totowa, NJ, 15-32. https://doi.org/10.1007/978-1-62703-278-0_2

[6] Robertson, D. (2003) Vigs Vectors for Gene Silencing: Many Targets, Many Tools. *Annual Review of Plant Biology*, **55**, 495-519. https://doi.org/10.1146/annurev.arplant.55.031903.141803

[7] Scofield, S.R. and Nelson, R.S. (2009) Resources for Virus-Induced Gene Silencing in the Grasses. *Plant Physiology*, **149**, 152-157. https://doi.org/10.1104/pp.108.128702

[8] Senthil-Kumar, M. and Mysore, K.S. (2011) Virus-Induced Gene Silencing Can Persist for more than 2 Years and also Be Transmitted to Progeny Seedlings in *nicotiana benthamiana* and Tomato. *Plant Biotechnology Journal*, **9**, 797-806. https://doi.org/10.1111/j.1467-7652.2011.00589.x

[9] Waterhouse, P.M. and Helliwell, C.A. (2003) Exploring Plant Genomes by RNA-Induced Gene Silencing. *Nature Reviews Genetics*, **4**, 29-38. https://doi.org/10.1038/nrg982

[10] Lange, M., Yellina, A.L., Orashakova, S. and Becker, A. (2013) Virus-Induced Gene Silencing (VIGS) in Plants: An Overview of Target Species and the Virus-Derived Vector Systems. Virus-Induced Gene Silencing. Humana Press, Totowa, NJ.

[11] Lindbo, J.A., Fitzmaurice, W.P. and Dellacioppa, G. (2001) Virus-Mediated Reprogramming of Gene Expression in Plants. *Current Opinion in Plant Biology*, **4**, 181-185. https://doi.org/10.1016/S1369-5266(00)00158-8

[12] Stewart, C.N. (2011) Plant Transformation Technologies. Blackwell Publishing Ltd., Hoboken, NJ. https://doi.org/10.1002/9780470958988

[13] Peele, C., Jordan, C.V., Muangsan, N., Turnage, M., Egelkrout, E., Eagle, P., *et al.* (2001) Silencing of a Meristematic Gene Using Geminivirus-Derived Vectors. *The Plant Journal*, **27**, 357-366. https://doi.org/10.1046/j.1365-313x.2001.01080.x

[14] Ramegowda, V., Mysore, K.S. and Senthilkumar, M. (2014) Virus-Induced Gene Silencing Is a Versatile Tool for Unraveling the Functional Relevance of Multiple Abiotic-Stress-Responsive Genes in Crop Plants. *Frontiers in Plant Science*, **5**, 323. https://doi.org/10.3389/fpls.2014.00323

[15] Senthil-Kumar, M., Gowda, H.V.R., Hema, R., Mysore, K.S. and Udayakumar, M. (2008) Virus-Induced Gene Silencing and Its Application in Characterizing Genes Involved in Water-Deficit-Stress Tolerance. *Journal of Plant Physiology*, **165**, 1404-1421. https://doi.org/10.1016/j.jplph.2008.04.007

[16] Kumagai, M. H., Donson, J., Dellacioppa, G., Harvey, D., Hanley, K. and Grill, L.K. (1995) Cytoplasmic Inhibition of Carotenoid Biosynthesis with Virus-Derived RNA. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 1679-1683. https://doi.org/10.1073/pnas.92.5.1679

[17] Himber, C., Dunoyer, P. and Moissiard, G. (2003) Transitivity-Dependent and -Independent Cell-to-Cell Movement of Rna Silencing. *EMBO Journal*, **22**, 4523-4533. https://doi.org/10.1093/emboj/cdg431

[18] Ratcliff, F., Harrison, B.D. and Baulcombe, D.C. (1997) A Similarity between Viral Defense and Gene Silencing. *Science*, **276**, 1558-1560. https://doi.org/10.1126/science.276.5318.1558

[19] Ratcliff, F., Ana Montserrat Martin-Hernandez and Baulcombe, D.C. (2001) Technical Advance: Tobacco Rattle Virus as a Vector for Analysis of Gene Function by Silencing. *The Plant Journal*, **25**, 237-245. https://doi.org/10.1046/j.0960-7412.2000.00942.x

[20] Liu, Y., Schiff, M. and Dinesh-Kumar, S.P. (2002) Virus-Induced Gene Silencing in Tomato. *The Plant Journal*, **31**, 777-786. https://doi.org/10.1046/j.1365-313X.2002.01394.x

[21] Holzberg, S., Brosio, P., Gross, C. and Pogue, G.P. (2002) *Barley Stripe Mosaic Virus*-Induced Gene Silencing in a Monocot Plant. *The Plant Journal*, **30**, 315-327. https://doi.org/10.1046/j.1365-313X.2002.01291.x

[22] Noueiry, A.O. and Ahlquist, P. (2003) Brome Mosaic Virus RNA Replication: Revealing the Role of the Host in RNA Virus Replication. *Annual Review of Phytopathology*, **41**, 77-98. https://doi.org/10.1146/annurev.phyto.41.052002.095717

[23] Kroner, P.A., Young, B.M. and Ahlquist, P. (1990) Analysis of the Role of Brome Mosaic Virus 1a Protein Domains in RNA Replication, Using Linker Insertion Mutagenesis. *Journal of Virology*, **64**, 6110-6120.

[24] Quadt, R. and Jaspars, E.M.J. (1990) Purification and Characterization of Brome Mosaic Virus RNA-Dependent RNA Polymerase. *Virology*, **178**, 189-194. https://doi.org/10.1016/0042-6822(90)90393-6

[25] Kao, C.C. and Ahlquist, P. (1992) Identification of the Domains Required for Direct Interaction of the Helicase-Like and Polymerase-Like RNA Replication Proteins of Brome Mosaic virus. *Journal of Virology*, **66**, 7293-7302.

[26] Dasgupta, R. and Kaesberg, P. (1982) Complete Nucleotide Sequences of the Coat Protein Messenger RNAs of Brome Mosaic Virus and Cowpea Chlorotic Mottle Virus. *Nucleic Acids Research*, **10**, 703-713. https://doi.org/10.1093/nar/10.2.703

[27] Ahlquist, P., Dasgupta, R. and Kaesberg, P. (1984) Nucleotide Sequence of the Brome Mosaic Virus Genome and Its Implications for Viral Replication. *Journal of Molecular Biology*, **172**, 369-383. https://doi.org/10.1016/S0022-2836(84)80012-1

[28] Mise, K. and Ahlquist, P. (1995) Host-Specificity Restriction by Bromovirus Cell to Cell Movement Protein Occurs after Initial Cell-to-Cell Spread of Infection in Nonhost Plants. *Virology*, **206**, 276-286. https://doi.org/10.1016/S0042-6822(95)80043-3

[29] Schmitz, I. and Rao, A.L. (1996) Molecular Studies on Bromovirus Capsid Protein. I. Characterization of Cell-to-Cell Movement-Defective RNA3 Variants of Brome Mosaic Virus. *Virology*, **226**, 281-293. https://doi.org/10.1006/viro.1996.0656

[30] Ding, X., Ballard, K. and Nelson, R. (2010) Improving Virus Induced Gene Silencing (VIGS) in Rice through Agrobacterium Infiltration. In: Antoun, H., Avis, T., Brisson, L., Prevost, D. and Trepanier, M., Eds., *Biology of Plant-Microbe Interactions*, *International Society for Molecular Plant-Microbe Interactions*, St. Paul, MN. Paper No. 59.

[31] Ding, X.S., Schneider, W.L., Chaluvadi, S.R., Mian, M.A. and Nelson, R.S. (2006) Characterization of a *Brome Mosaic Virus* Strain and Its Use as a Vector for Gene Silencing in Monocotyledonous Hosts. *Molecular Plant Microbe Interactions*, **19**, 1229-1239. https://doi.org/10.1094/MPMI-19-1229

[32] Pacak, A., Strozycki, P.M., Barciszewska-Pacak, M., Alejska, M., Lacomme, C., Jarmołowski, A., Szweykowska-Kulinska, Z. and Figlerowicz, M. (2010) The Brome Mosaic Virus-Based Recombination Vector Triggers a Limited Gene Silencing Response Depending on the Orientation of the Inserted Sequence. *Archives of Virology*, **155**, 169-179. https://doi.org/10.1007/s00705-009-0556-9

[33] Sun, L., Zhang, H., Li, D., Huang, L., Hong, Y., Ding, X.S., Nelson, R.S., Zhou, X. and Song F. (2013) Functions of Rice NAC Transcriptional Factors, ONAC122 and ONAC131, in Defense Responses against *Magnaporthe grisea*. *Plant Molecular Biology*, **81**, 41-56. https://doi.org/10.1007/s11103-012-9981-3

[34] Donaire, L., Barajas, D., Martínez-García, B., Martínez-Priego, L., Pagán, I. and Llave, C. (2008) Structural and Genetic Requirements for the Biogenesis of Tobacco Rattle Virus-Derived Small Interfering RNAs. *Journal of Virology*, **82**, 5167-5177. https://doi.org/10.1128/JVI.00272-08

[35] Senthilkumar, M. and Mysore, K.S. (2014) Tobacco Rattle Virus-Based Virus-Induced Gene Silencing in *Nicotiana benthamiana*. *Nature Protocols*, **9**, 1549-1562. https://doi.org/10.1038/nprot.2014.092

[36] MacFarlane, S.A. (1999) Molecular Biology of the Tobraviruses. *Journal of General Virology*, **80**, 2799-2807. https://doi.org/10.1099/0022-1317-80-11-2799

[37] Bruce, G., Gu, M., Shi, N., Liu, Y. and Hong, Y. (2011) Influence of Retinoblastoma-Related Gene Silencing on the Initiation of DNA Replication by African Cassava Mosaic Virus Rep in Cells of Mature Leaves in *Nicotiana benthamiana* Plants. *Virology Journal*, **8**, 561. https://doi.org/10.1186/1743-422X-8-561

[38] Turnage, M.A., Muangsan, N., Peele, C.G. and Robertson, D. (2010) Geminivirus-Based Vectors for Gene Silencing in Arabidopsis. *Plant Journal for Cell & Molecular Biology*, **30**, 107-114. https://doi.org/10.1046/j.1365-313X.2002.01261.x

[39] Gosselé, V., Faché, I., Meulewaeter, F., Cornelissen, M. and Metzlaff, M. (2002) SVISS—A Novel Transient Gene Silencing System for Gene Function Discovery and Validation in Tobacco Plants. *The Plant Journal*, **32**, 859-866. https://doi.org/10.1046/j.1365-313X.2002.01471.x

[40] Choong-Min, R., Anand, A., Kang, L. and Mysore, K.S. (2004) Agrodrench: A Novel and Effective Agroinoculation Method for Virus-Induced Gene Silencing in Roots and Diverse Solanaceous Species. *The Plant Journal*, **40**, 322-331. https://doi.org/10.1111/j.1365-313X.2004.02211.x

[41] Jeyabharathy, C., Shakila, H. and Usha, R. (2015) Development of a VIGS Vector Based on the β-Satellite DNA Associated with Bhendi Yellow Vein Mosaic Virus. *Virus Research*, **195**, 73-78. https://doi.org/10.1016/j.virusres.2014.08.009

[42] Liu, Y. (2010) Tobacco *Rar*1, *EDS*1 and *NPR*1/*NIM*1 Like Genes Are Required for *N*-Mediated Resistance to Tobacco Mosaic Virus. *Plant Journal*, **30**, 415-429. https://doi.org/10.1046/j.1365-313X.2002.01297.x

[43] Liu, E. and Page, J.E. (2008) Optimized cDNA Libraries for Virus-Induced Gene Si-

lencing (VIGS) Using Tobacco Rattle Virus. *Plant Methods*, **4**, 5. https://doi.org/10.1186/1746-4811-4-5

[44] Scofield, S.R., Huang, L., Brandt, A.S. and Gill, B.S. (2005) Development of a Virus-Induced Gene-Silencing System for Hexaploid Wheat and Its Use in Functional Analysis of the *Lr*21-Mediated Leaf Rust Resistance Pathway. *Plant Physiology*, **138**, 2165-2173. https://doi.org/10.1104/pp.105.061861

[45] Wang, X., Cao, A., Yu, C., Wang, D., Wang, X. and Chen, P. (2010) Establishment of an Effective Virus Induced Gene Silencing System with BSMV in *Haynaldia villosa*. *Molecular Biology Reports*, **37**, 967. https://doi.org/10.1007/s11033-009-9766-1

[46] Várallyay, É., Giczey, G. and Burgyán, J. (2012) Virus-Induced Gene Silencing of *Mlo* Genes Induces Powdery Mildew Resistance in *Triticum aestivum*. *Archives of Virology*, **157**, 1345-1350. https://doi.org/10.1007/s00705-012-1286-y

[47] Delventhal, R., Zellerhoff, N. and Schaffrath, U. (2011) Barley Stripe Mosaic Virus-Induced Gene Silencing (BSMV-IGS) as a Tool for Functional Analysis of Barley Genes Potentially Involved in Nonhost Resistance. *Plant Signaling & Behavior*, **6**, 867-869. https://doi.org/10.4161/psb.6.6.15240

[48] Hein, I., Barciszewskapacak, M., Hrubikova, K., Williamson, S., Dinesen, M., Soenderby, I.E., *et al.* (2005) Virus-Induced Gene Silencing-Based Functional Characterization of Genes Associated with Powdery Mildew Resistance in Barley. *Plant Physiology*, **138**, 2155-2164. https://doi.org/10.1104/pp.105.062810

[49] Bahieldin, A., Atef, A., Edris, S., Gadalla, N.O., Ali, H.M., Hassan, S.M., *et al.* (2016) Ethylene Responsive Transcription Factor ERF109 Retards PCD and Improves Salt Tolerance in Plant. *BMC Plant Biology*, **16**, 216. https://doi.org/10.1186/s12870-016-0908-z

[50] Jin, J.H., Zhang, H.X., Tan, J.Y., Yan, M.J., Li, D.W., Khan, A., *et al.* (2015) A New Ethylene-Responsive Factor CaPTI1 Gene of Pepper (*Capsicum annuum* L.) Involved in the Regulation of Defense Response to *Phytophthora capsici*. *Frontiers in Plant Science*, **6**, 1217. https://doi.org/10.3389/fpls.2015.01217

[51] Mishra, S., Phukan, U.J., Tripathi, V., Singh, D.K., Luqman, S. and Shukla, R.K. (2015) PsAP2 an AP2/ERF Family Transcription Factor from *Papaver somniferum* Enhances Abiotic and Biotic Stress Tolerance in Transgenic Tobacco. *Plant Molecular Biology*, **89**, 173-186. https://doi.org/10.1007/s11103-015-0361-7

[52] Li, C.P. (2011) Analysis of Drought Resistance and Water Saving Function of S-Adenosine Methionine Metabolic Pathway Related Genes in Wheat. Doctoral Dissertation, Northwest University of Agriculture and Forestry Technology, Xianyang.

[53] Bennypaul, H.S., Mutti, J.S., Rustgi, S., Kumar, N., Okubara, P.A. and Gill, K.S. (2012) Virus-Induced Gene Silencing (VIGS) of Genes Expressed in Root, Leaf, and Meiotic Tissues of Wheat. *Functional & Integrative Genomics*, **12**, 143-156. https://doi.org/10.1007/s10142-011-0245-0

[54] Oikawa, A., Rahman, A., Yamashita, T., Taira, H. and Kidou, S. (2007) Virus-Induced Gene Silencing of p23k in Barley Leaf Reveals Morphological Changes Involved in Secondary Wall Formation. *Journal of Experimental Botany*, **58**, 2617-2625. https://doi.org/10.1093/jxb/erm109

[55] Kawalek, A., Dmochowska-Boguta, M., Nadolska-Orczyk, A. and Orczyk, W. (2012) A New BSMV-Based Vector with Modified β Molecule Allows Simultaneous and Stable Silencing of Two Genes. *Cellular & Molecular Biology Letters*, **17**, 107-123. https://doi.org/10.2478/s11658-011-0041-9

[56] Liu, G., Wu, Y., Xu, M., Gao, T., Wang, P., Wang, L., *et al.* (2016) Virus-Induced

Gene Silencing Identifies an Important Role of the Ta*RSR*1 Transcription Factor in Starch Synthesis in Bread Wheat. *International Journal of Molecular Sciences*, **17**, 1557. https://doi.org/10.3390/ijms17101557

[57] Furbank, R.T., Parry, N.M.A., Reynolds, M., Chapman, J.C., Manès, Y., Bonnett, D., *et al.* (2011) Raising Yield Potential of Wheat. I. Overview of a Consortium Approach and Breeding Strategies. *Journal of Experimental Botany*, **62**, 439-452. https://doi.org/10.1093/jxb/erq311

[58] Zhang, N., Zhang, L., Shi, C., Zhao, L., Cui, D. and Chen, F. (2018) Identification of Proteins Using iTRAQ and Virus-Induced Gene Silencing Reveals Three Bread Wheat Proteins Involved in the Response to Combined Osmotic-Cold Stress. *Journal of Proteome Research*, **17**, 2256-2281. https://doi.org/10.1021/acs.jproteome.7b00745

[59] Zaidi, S.A. and Mansoor, S. (2017) Viral Vectors for Plant Genome Engineering. *Frontiers in Plant Science*, **8**, e1044191. https://doi.org/10.3389/fpls.2017.00539

[60] Cheuk, A. and Houde, M. (2017) A New Barley Stripe Mosaic Virus Allows Large Protein Overexpression for Rapid Function Analysis. *Plant Physiology*, **176**, Article ID: 01412. https://doi.org/10.1104/pp.17.01412

[61] Zhang, J., Yu, D., Zhang, Y., Liu, K., Xu, K., Zhang, F., *et al.* (2017) Vacuum and Co-Cultivation Agroinfiltration of (Germinated) Seeds Results in Tobacco Rattle Virus (TRV) Mediated Whole-Plant Virus-Induced Gene Silencing (VIGS) in Wheat and Maize. *Frontiers in Plant Science*, **8**, e0145227. https://doi.org/10.3389/fpls.2017.00393

[62] Godge, M.R., Purkayastha, A., Dasgupta, I. and Kumar, P.P. (2008) Virus-Induced Gene Silencing for Functional Analysis of Selected Genes. *Plant Cell Reports*, **27**, 209-219. https://doi.org/10.1007/s00299-007-0460-2

[63] Li, C. (2011) Development and Application of a High Throughput Barley Stripe Mosaic Virus Vector for Virus Induced Gene Silencing in Monocots and Dicots. Doctoral Dissertation, China Agricultural University, Guangzhou.