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The Current Status and Prospects of Virus-Induced Gene Editing in Plant

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Abstract

This report mainly reviews the current status of virus-induced gene editing (VIGE). Viral-vector types and parameters affecting the efficacy of VIGE-based gene editing in plant, and prospects will be addressed.

Keywords: Virus-Induced Gene Editing (VIGE); Plant viral Vectors, CRISPR/Cas; sgRNA; Somatic editing; Heritable editing

Abbreviation: VIGS: Virus-Induced Gene Editing; VIGE-Cas9: VIGE-nonGM; FT: Flowering Locus T; RPS5A: Ribosomal protein S5A; TRV: Tobacco Rattle Virus; BSMV: Barley stripe mosaic virus; CLCrV: Cotton Leaf Crumple Virus; CaLCuV: Cabbage Leaf Curl virus; TMV: Tobacco Mosaic Virus; BNYVV: Beet Necrotic Yellow Vein Virus; FoMV: Foxtail Mosaic Virus; PVX: Potato Virus X; SYNV: Sonchus Yellow Net Rhabdovirus; TSWV: Tomato Spotted Wilt Virus; TBSV: Tomato Bushy Stunt Virus; TEV: Tobacco Etch Virus

Introduction

For many plant species, the biggest challenge is effectively delivering CRISPR/Cas reagents into plant cells for target gene editing. If this critical step can be overcome, gene editing efficiency will greatly improve. Currently, the most commonly used delivery method is the "transgene-mediated approach" for CRISPR/Cas reagents through Agrobacterium-mediated transformation or biolistic bombardment. After the expression vector with Cas9-sgRNA:target is transferred to the plant genome, the expressed Cas9 protein is guided to the plant genome via sgRNA. Edit the target region (near PAM) to generate InDels mutations. Although many plants have developed this system, their efficiency is low, especially for non-model plant species. Consequently, more progeny-containing transgene constructs must be screened. Transformation technical difficulties and the subsequent breeding of edited progeny to eliminate transgenes are labor-intensive, time- and space-consuming. Therefore, developing "non-GM" genome editing strategies that bypass tissue culture and regeneration processes can quickly improve the effectiveness of gene editing. VIGE (virus-induced genome editing) is one of these strategies. Plant viruses have been modified, recently, for VIGE (reviewed in [1]). For VIGE, the viral vectors carrying the sgRNA::target and related constructs were agroinfiltration onto plant tissues(or alternative methods such as spraying the leaves or soaking the roots or floral dip, etc.) for gene editing (reviewed in S [2]).

Types of VIGE Systems

VIGE has two types, including VIGE-Cas9 and VIGE-nonGM strategies. Viral vectors suitable for VIGE are well addressed and reviewed in [3]. The following introduces updated information on examples of successful VIGE research based on VIGE strategies and types of viral vectors.

VIGE-Cas9 strategy

The VIGE-Cas9 strategy uses Agrobacteria harboring viral vectors with the sgRNA:target and agroinfiltration onto leaves of a "Cas9-transgenic plant".

Somatic editing

Several factors would affect somatic editing efficacy including viral vectors, RNA mobile elements (FTs, tRNAs), RNA silencing suppressors, promoters of Cas9 transgene, and environmental factors. First, the somatic editing of VIGE would be greatly

affected by the types of viral vectors and their target host plants. VIGE systems of most viral vectors are commonly established in *Nicotiana benthamiana*, a model plant species, that hosts many plant viruses. In Cas9-transgenic *N. benthamiana*, the somatic editing efficiency of VIGE in systemic leaves was high (>50%) in TRV, CalCuV, BYNNV, and PVX indicating systemic infection and expression sgRNAs were well established in these VIGE-viral vectors [4-8]. For TMV-based VIGE, high editing efficiency (70%) only detected transitionally in inoculated leaves but not systemic leaves partly because of the severe symptoms or strong plant RNA silencing effect which makes it, not an ideal vector of choice [1,9]. With the aid of P19, an RNA silencing suppressor, the editing efficiency in inoculated leaves was higher than that of control but still no editing results from systemic leaves were shown [9]. For BYNNV-based VIGE, only a mild strain Hu3 replaced the severe strain as the VIGE vector could obtain better somatic editing results (78%) in systemic leaves of Cas9-transgenic *N. Benthamiana* [7]. For Arabidopsis, another model plant, somatic editing of two target loci was higher in PEBV (57%-63%) than that of TRV (27%-35%) [10]. For tomato, higher somatic editing efficiency was reported in TRV (57%-94%) [11,12] than that in PVX (19.46-47%). The cases of the same viral vectors showed different efficiency on hosts, for example, BSMV showed higher efficiency in systemic leaves of wheat than that of maize [13]. For FoMV-based VIGE, somatic editing efficiency was higher in *Setaria viridis* (60%) than in *N. benthamiana* (0-8%) [14], and low or no systemic editing was detected in maize [15].

For the VIGE-Cas9 system, the 35S promoter is widely applied for overexpression of Cas9 at target plant species and is commonly called a Cas9-OE transgenic plant. This could ensure Cas9 potentially expressing in every cell of the transgenic plants to provide CRISPR editing. However, Cas9-OE is not always the best choice. For CLCrV-based VIGE, somatic editing in 35S::Cas9-trangenic Arabidopsis plants showed an editing efficiency of 18.75% for two target loci, however, a transgenic Cas9 driven by meristem-expression promoter Yao (Yao::Cas9) plants showed much higher editing efficiency (50%-62.5%) [16]. For the Environmental factors, heat treatment was shown the increase TRV-based VIGE somatic editing from 40.40% (the control) to 57.3% (37°C and 12 hours) in Cas9-OE MicroTom tomato [12].

Heritable editing

Heritable editing was obtained in two ways including seed transmission and alternatively regeneration through tissue culture derived from infected leaves of edited plants. Those viruses having the capability to invade meristems including TRV, PEBV, BSMV, CLCrV could obtain heritable editing through the selfing progeny of VIGE-edited M0 plants. However, for those that could not infect meristems including CaLCuV, TMV, BYNNV, FoMV, and PVX, heritable editing could only obtained from the regeneration through tissue culture of infected leaves of VIGE-edited M0 plants. Factors affecting heritable editing efficacy are RNA mobile elements (FTs, tRNAs), promoters of Cas9 transgene, and environmental factors.

Seed Transmission: The RNA mobile elements (FTs, tRNAs) were shown to increase the chance of the virus infecting meristemic tissues. Several studies have tested their effect on VIGE editing efficiency. In a TRV-based VIGE in Cas9-OE *N. benthamiana* study, FTs, and RNAs did show an increase in heritable editing (up to 65%) and no significant difference among different FT variants [5]. In TRV-based VIGE of base editing experiments, $tRNA^{Ile}$ at 3'end of sgRNA did increase the heritable editing in Cas9-OE Arabidopsis [17]. This study also showed that environmental factors influencing flowering such as long days and low temperature also showed increasing heritable base editing efficiency in selfing progeny. In BSMV-based editing on Cas9-OE wheat, the heritable editing efficiency of the sgRNA constructs without FT or tRNA was 0.8% and increased to 2.3% and 3.00% with mTAFT (the wheat FT homolog) and $tRNA^{leu}$ [18]. In Yao::Cas9-transgenic Arabidopsis, the CLCrV-based VIGE construct adding FT at 5'end of sgRNA showed higher heritable editing efficiency(4.35%-8.79%) than the control (0%) [16]. In TRV-based VIGE on wild tobacco (*N. attenuate*), 35S::Cas9-trangenic plants showed no edited progeny recovered, however, a transgenic Cas9 driven by meristem-expression promoter RPS5A(RPS5A::Cas9) plants showed higher heritable editing efficiency (2.4%) [19].

Regeneration through Tissue Culture: For CaLCuV- and BYNNV-based VIGE, somatic editing efficiencies were high, but no heritable editing by regeneration from tissue culture was attempted. In the FoMV-based VIGE on *N. benthaminana, Setaria viridis*, and *Zea maize*, heritable editing through seeds was attempted, however, none of them could recover any edited progeny [14,15]. In PVX-based VIGE, heritable editing by regeneration from tissue culture was shown to have a high efficiency for targeting PDS (70%) and SGR1(60%) in Cas9-OE MicroTom tomato [20].

VIGE-nonGM Strategy

VIGE-nonGM strategy uses an Agrobacterium with viral vectors carrying "Cas9 and sgRNA" construct and agroinfiltration onto leaves of wild-type (non-transgenic) plants. Currently, only a few viral vectors were developed for VIGE-nonGM, because of the limitation of cargo capacity and the stability for the expression of Cas and sgRNAs of viral vectors.

Cargo Capacity

When the large-size DNA insert is carried by the viral vector, it is easy to fall off during the replication and spreading of viruses in host plants. Most plant viral vectors are too small to carry the large inserts as the size of CRISPR/Cas9 reagents (Cas9 and sgRNA > 4.5 kb and >6 kb for base editor) and consequently could not be suitable as a viral vector for VIGE-nonGM system. However, some of the viral vectors have been shown successfully developed. SYNV with rod-like virion structure, TSWV reconstructed to

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flexible helical nucleocapsids with helical symmetry and PVX with filamentous flexible structure make them could accommodate larger genetic cargoes [21-23].. Alternatively, to increase viral vectors suitable for VIGE-nonGM system, Cas proteins such as Cas12 variants with a size smaller than Cas9 would be able to fit many viral vectors ([24,25] reviewed in [26]). For example, the most popular viral vector TRV with a wide host range, mild symptoms on hosts, and seed-inheritable qualities.

Somatic and heritable editing

In SYNV-based VIGE, the somatic editing efficiency of several loci in *N. benthamiana* was high in systemic leaves (40%-91%) and heritable editing (57%-93%) could be successfully generated through tissue culture of infected leaves [21]. In TSWV-based VIGE, somatic editing efficiency of 3 loci in *N. benthamiana* was 26.4%-68.1%% (for constructs Cas12a or Cas9 with sgRNAs), 63.5% in *N. tobacum* and 78.2% in tomato. In addition, base editing (up to 6 kb) was also successfully achieved including ABE 27.7%-38.6% and CBE 55%-81.8% in *N. benthamiana* and ABE 15%-65% and CBE 29%-95% for *N. tobacum* and tomato. Heritable editing was achieved at 16%-18% and 40% in *N. tobacum* and tomato [22]. This could increase to 30%-38% and 68.8% after ribavirin treatment supplemented in tissue culture media. In addition, TSWV-based VIGE was also shown successfully developed in pepper with somatic editing efficiency of 57.65%-75.73% and heritable editing of 77.9% [27]. In PVX-based VIGE, somatic editing efficiency of 22%-100% in *N. benthamiana*, eggplant, and 3 susceptible cultivars of potato. Heritable editing was high (60%) in *N. benthamiana* but low in tomato (1.7%-12.4%) and potato (30.50%) [23,12]. Multiplexing of VIGE-nonGM was achieved in SYNV- and TSWV-based VIGE [21,22].

RNA Mobile Element (tRNAs) and RNA Silencing Suppressors

It is reported that tRNAs were added to SYNV-based constructs to increase editing efficiency [21]. The RNA silencing suppressors including TBSV P19, BSMV γb, and TEV P1/Hc-Pro were also co-agroinfiltration along with TSWV-based VIGE vectors on target plants to increase virus spreading and editing efficiency [22,27].

Pathogenicity

TSWV-based VIGE showed severe symptoms in plants and a toxic effect on regeneration which would affect greatly somatic and heritable editing efficiency. Liu et al. [22] employed ribavirin treatment and overcame this problem. Interestingly, TSWV-based VIGE did not show virulence and toxic effects on plants and the regeneration process in pepper [27]. This indicates that the infected symptoms caused by the same type of virus would not be consistent on different hosts or the same plant species of different cultivars. Viral vectors should be screened thoroughly among individual lines, cultivars, and species to decide if it is suitable for VIGE vectors.

Host Plants

For the VIGE-Cas9 system, TRV has a wide host range including most dicot and some monocot plant species and a PVX-based vector would be an efficient editing tool for Solanaceae crops (reviewed in [2]). Consequently, both of them are very popular tools for plant functional genetics. The PEBV-based VIGE is suitable especially for legumes [10]. BYNNV-based VIGE would be an ideal editing tool for *Beta vulgaris, Tetragania expansa, Chenopodium quinoa,* and *Spinacea oleracea* [7]. TSWV has a very wide host range including 1090 plant hosts and would be valuable for VIGE editing to trait improvement for many economically important crops, especially for horticultural and ornamental plants [28]. For monocots, FoMV-based VIGE was attempted because the host of this viral vector was favorable for Poaceae. However, it was shown very low efficiency in *N. benthamiana* even with the aid of P19 [29]. This indicates that a lot more studies are needed to develop viral vectors for VIGE-nonGM on monocot crops.

System Comparison

The VIGE-nonGM system would be the best choice since it is completely transgene-free and any host plants can be edited. The limitation of this system is only a few viral vectors are currently developed and they all need to be regenerated through tissue culture to obtain stably edited progeny lines. For other systems, the "transgene-mediated approach" and VIGE-Cas9 system to perform gene editing, they all need to go through the difficulty of plant regeneration by tissue culture and frequently have very low efficiency for obtaining transgenic plants. Still, comparing individual sgRNAs need to go through the agrobacterium-mediated transformation and tissue culture in a "transgene-mediated approach", the VIGE-Cas9 system greatly improves the editing efficiency. Because once the Cas9-transgenic line is established, there is no need to carry out transformation and tissue culture processes for individual sgRNAs designed from different gene targets, instead, viral vectors carrying different sgRNAs can be agroinfiltration onto plants to quickly obtain editing results within weeks. Most importantly, many viral vectors for the VIGE-Cas9 system have been developed for VIGE editing a wide range of host plant species.

For example, TRV, the vector of choice, has many advantages over other viral vectors. TRV has a wide host range including many important crops (reviewed in [2]). TRV-based VIGE showed gene editing successfully on Cas9-trangenic lines of plant species including *N. benthaminia*, Arabidopsis, *N. attenuate*, tomato cv. Ailsa Craig, and tomato cv. MicroTom etc [4,5,10-12,19,30]. Not only somatic but also germline editing efficiency was high with or without the aid of mobile RNA elements [5,10,11,19]. In addition, TRV-based VIGE in base editing and epigenomic editing were developed successfully [17,31]. Importantly, no TRV virus or vector-related DNA was found in the edited progeny [5] and

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no off-target mutations detected were also demonstrated [4,10]. However, the need for Cas9-transgenic lines should be the limit of this system. A small Cas protein along with sgRNA should be able to fit inside the pTRV2 vector to develop a TRV-based VIGE-nonGM system in the future.

Conclusion

In VIGE, no off-target editing detected [4,10], no virus or viral vectors were detected in the edited progeny was demonstrated, and healthy virus-free edited plants were developed successfully (TRV in 5; PVX in 20; TSWV in 22). In addition, multiplexing several sgRNAs in the same viral-vector construct was shown successfully in TRV- [30,31], BSMV-based [13,18,32], TMV-based [1], SYNV-based [21] and TSWV- based [22] VIGE. These indicate a great advantage of the VIGE system and do provide strong evidence that VIGE systems are a simple, efficient, and time- and labor-cost-saving tool for plant genome editing.

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