Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

Generation of novel resistance genes using mutation and targeted gene editing

Amit Gal-On[1], Marc Fuchs[2] and Stewart Gray[3,4]

Classical breeding for virus resistance is a lengthy process and is restricted by the availability of resistance genes. Precise genome editing is a 'dream technology' to improve plants for virus resistance and these tools have opened new and very promising ways to generate virus resistant plants by disrupting host susceptibility genes, or by increasing the expression of viral resistance genes. However, precise targets must be identified and their roles understood to minimize potential negative effects on the plant. Nonetheless, the opportunities for genome editing are expanding, as are the technologies to generate effective and broad-spectrum resistance against plant viruses. Here we provide insights into recent progress related to gene targets and gene editing technologies.

Addresses
[1] Departments of Plant Pathology and Weed Research, ARO, Volcani Center, Bet Dagan 50250, Israel
[2] Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, United States
[3] Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, United States
[4] Departments of Emerging Pest and Pathogen Research Unit, USDA, ARS, Ithaca, NY 14853, United States

Corresponding author: Gal-On, Amit (amitg@volcani.agri.gov.il)

Current Opinion in Virology 2017, **26**:98–103

This review comes from a themed issue on **Engineering for viral resistance**

Edited by **John Carr** and **Peter Palukitis**

http://dx.doi.org/10.1016/j.coviro.2017.07.024

1879-6257/Published by Elsevier B.V.

Introduction

Despite the explosion in discovery and characterization of susceptibility and resistance genes that regulate plant virus infection, useful resistance for many host–virus combinations remains elusive. However, the increased understanding of host–virus interactions and natural allele variation has provided gene targets that can be transferred into or modified in plant species to create resistance where natural resistance alleles have not been found. Natural plant genes can be modified to either gain resistance or to lose susceptibility to a virus. Exploiting natural gene variation and their contributions to resistance or susceptibility in combination with new gene editing technologies has allowed the creation of resistance to important virus families in numerous staple and specialty crops. This has been especially important for generating variants of host nucleases that can attack DNA plant viruses and variants of host transcription factors that are no longer useable for RNA viruses.

Genome editing is based on double-strand breaks at specific sites, which can be repaired by non-homologous end-joining via the cell autonomous mechanism, leading to indels (mutations of insertion/deletion of nucleotides). Alternatively, editing can be based on homologous recombination by which homologous DNA molecules swap nucleotide sequences within the double-strand break [1,2••]. Precise genome editing has been successfully developed in plants with artificial nuclease Zinc Finger Nucleases [3] and continued with meganucleases and Transcription Activator Like Effector Nucleases (TALENs). Such nucleases target the DNA at specific sites, and DNA cleavage made by the fused restriction enzyme FokI. Clustered Regularly Interspaced Short Palindromic Repeats along with the associated protein 9 nuclease (CRISPR/Cas9) and single guide-RNA is the latest breakthrough technology in eukaryote genome editing [4,5] with rice being the first edited plant genome [6]. Off-target editing is an obstacle in precise genome editing, however the use of different types of Cas proteins and two target sequences can reduce such problems. In any event, genome editing technologies have been extensively deployed to engineer resistance to viruses in plants.

Modifying plant genes for gain of resistance

The recent advent of genome editing through the use of programmable nucleases opened new avenues for engineering resistance to viruses in crops [7••]. Since nucleases target DNA, most studies in plants edited for virus resistance to date involved the development of geminivirus resistance by direct targeting of the geminivirus genome. Initial applications of genome editing technologies to confer virus resistance in transgenic plants focused on zinc finger nucleases to target the replication-associated protein of beet severe curly top virus [8]. In another study, nuclease-targeting a conserved 25 bp *Rep* region protected tomato against tomato yellow leaf curl China virus and tobacco curly shoot virus [9]. TALENs were also used as a platform for designing broad-spectrum resistance to begomoviruses by targeting two highly

conserved viral genomic regions; that is, the replication-associated AC1 and intergenic nonanucleotide sequence [10]. More recently, CRISPR/Cas9 was deployed to confer resistance to geminiviruses in transgenic *Nicotiana benthamiana* expressing Cas9 and transient expression of guide RNA via a tobacco rattle virus vector [11••,12]. CRISPR-Cas containing a single-guide RNA was also designed against the *Rep* and *CP* genes, and the intergenic region of tomato yellow leaf curl virus and beet curly top virus targeted and degraded both viruses [12]. Also, single guide RNAs containing the conserved nonanucleotide sequences of the geminivirus origin of replication targeted and degraded both viruses, as well as merremia mosaic virus [12]. In another study, a transgenic plant harboring Cas9 and single guide RNAs designed from coding and noncoding regions of beet severe curly top virus specifically targeted and degraded this virus [13]. Similar results were obtained using guide RNAs from Rep motifs, Rep-binding site, hairpin, and the nonanucleotide sequence of bean yellow dwarf virus [14••]. In a follow-up study, the conserved nonanucleotide sequence was used to target six begomoviruses simultaneously [11••]. Recently it was demonstrated that the class 2 type VI CRISPR-Cas effector C2c2 from *Leptotrichia shahii* efficiently targeted phage RNA [15••]. This last example broadens the CRISPR-Cas technologies that can be utilized as new resistance tools are developed to target RNA plant viruses. It is noteworthy that development of viral resistance by direct targeting of the geminivirus genome is based on engineering transgenic plants to express the CRISPR nuclease together with a single guide RNA in the same cell. Therefore, the final product is transgenic. However, the foreign gene used for knockout of a susceptibility/recessive gene (see below) can be eliminated by segregation in a subsequent generation, as demonstrated in the *Cseif4e* mutant, and the final product is non-transgenic [16••]. Alternatively, transfection of protoplasts with a ribonucleoprotein (Cas-9 protein-sgRNA) in potato [17•] also produces a non-transgenic product.

Modifying plant genes for loss of susceptibility

Nearly half of the plant genes conferring resistance to plant viruses are inherited in a recessive manner and encode molecules utilized by the pathogen to complete its infection cycle [18••]. Although recessive resistance is not unique to virus resistance [19], it does appear to be a more common defense mechanism against viruses than against other pathogen types [20,21]. Furthermore, recessive resistance, in general, seems to be more durable and less strain specific than dominant forms of resistance [22].

Most of the identified and characterized recessive genes have been translation initiation factors, although not all [23,24•]. The most widely found gene encodes eIF4E, a component of the translation initiation complex that functions as a cap binding protein, as well as in the recruitment of other factors to the translation initiation complex. Importantly, eIF4E structure and function is highly conserved among all eukaryotes [25•]. eIF4E interacts with viral RNA directly [26] or through a viral protein bound to viral RNA. Potyviruses utilize the virus encoded 5′ terminal-bound protein known as the Viral Protein genome linked or VPg, that binds directly to eIF4E in a strain-specific manner. A VPg is associated with the RNA of most members of the families *Potyviridae* or *Secoviridae*, as well as some members of the genera *Polerovirus* or *Sobemovirus* [27]. The VPg is also found in members of the vertebrate infecting *Picornaviridae*, *Caliciviridae* and possibly the *Astroviridae*, and they appear to have parallel roles in the virus infection cycle as plant virus VPg proteins [28]. VPg proteins are intrinsically disordered and interact with multiple virus and host proteins. They are known as hub proteins and control many processes related to virus production and spread. They are found in various subcellular localities depending on their precursor forms and function [27].

The VPg-eIF4E interaction has been widely studied and has been reviewed [18••]. Germaine to this review is that interactions (or lack thereof) between these proteins are often associated with recessive resistance to potyviruses. Virus resistance alleles arise when natural eIF4E mutations disrupt its ability to interact with the VPg. This prevents translation of the viral genome, but does not interfere with host mRNA translation. The evolution of mutant *eIF4E* genes that function as virus resistance genes has occurred independently in multiple plant species [29]. Studies of the mutations in multiple *eIF4E* resistance alleles from pepper found that, while the mutations in each allele are unique, they are clustered in the region of the three-dimensional eIF4E structure that is predicted to be involved in VPg binding [22,30]. This series of alleles conferring strain-specific resistance suggests that coevolution between host and pathogen is focused around changes in potyviral VPg and pepper. Detection of positive selection in both of these genes appears to confirm this hypothesis [31,32].

Knowledge of the specifics of the VPg-eIF4E binding sites in pepper identified critical amino acids and opened opportunities to use biotechnology to create de novo resistant alleles in plant species lacking any natural variation in the eIF4E genes. Over-expression of a pepper resistance allele or modified versions of the susceptible pepper allele in tomato or potato provided broad-spectrum potyvirus resistance [33•,34••,35•]. Presumably, most of the eIF4E protein in transgenic plants is modified eIF4E that is unable to interact with VPg and facilitate virus translation, but it still functions in host translation. The limited endogenous susceptible eIF4E protein can still interact with potyviral VPg, but its relative scarcity greatly reduces the probability of interaction. These

Download English Version:

https://daneshyari.com/en/article/5546163

Download Persian Version:

https://daneshyari.com/article/5546163

Daneshyari.com