Spotlight

Engineering Plants for Geminivirus Resistance with CRISPR/Cas9 System

Syed Shan-e-Ali Zaidi,1,2 Shahid Mansoor,2 Zahir Ali,1 Manal Tashkandi,1 and Magdy M. Mahfouz1,∗

The CRISPR/Cas9 system is an efficient genome-editing platform for diverse eukaryotic species, including plants. Recent work harnessed CRISPR/Cas9 technology to engineer resistance to geminiviruses. Here, we discuss opportunities, emerging developments, and potential pitfalls for using this technology to engineer resistance against single and multiple geminivirus infections in plants.

Geminiviruses cause devastating crop losses that threaten food security [1]. The geminivirus genome consists of a circular, single-stranded DNA molecule that becomes a double-stranded DNA replication intermediate in the plant cell nucleus and encodes four to seven multifunctional proteins. Recent efforts to engineer geminivirus resistance in crops have used several strategies, including: (i) viral protein-mediated resistance, in which plants are engineered to express variants of virus-encoded proteins to decrease the efficiency of a native viral protein; (ii) non-viral protein-mediated resistance, in which host proteins that interact with the viral genome or proteins are mutated or deleted, to disrupt intermediates in viral replication or transcription; (iii) viral RNA-mediated resistance, in which viral RNA is targeted using the host RNA interference (RNAi) machinery; and (iv) host-derived resistance, in which natural resistance is boosted by overexpressing host resistance (R) genes [2]. However, geminiviruses evolve rapidly by recombination, component capture, and mutation, allowing these viruses to rapidly counter or evade these strategies [3]. Overcoming these challenges will require a durable resistance strategy.

Clustered regularly interspaced short palindromic repeats (CRISPRs)/CRISPR-associated 9 (Cas9) is a prokaryotic molecular immunity system against invading viruses and has been harnessed as a powerful tool for targeted genomic editing. Bacterial and archaeal genomes acquire short pieces of DNA, called spacers, from invading viruses and incorporate these spacers within their genome, where they serve as a form of molecular memory. During subsequent infections, spacers are transcribed as part of the CRISPR array; after transcription and maturation, CRISPR RNA guides the Cas9 endonuclease to scan invading DNA and cleave the target sequence [4] at a site preceding the protospacer-associated motif (PAM), a trinucleotide sequence that is recognized by Cas9 and necessary for its binding to target DNA. The presence of the PAM enables the CRISPR/Cas9 machinery to differentiate between self and non-self DNA [4]. For targeted modification of genomic sites, transgenic production of a single guide RNA (sgRNA) provides specificity to the Cas9 endonuclease, allowing targeted cleavage of specific DNA sequences in eukaryotic cells. Recent work used the CRISPR/Cas9 system for targeted improvement of crop traits [5].

Three recent studies demonstrated that the CRISPR/Cas9 system could be harnessed to confer resistance against geminiviruses in plants by using sgRNAs designed to target viral genomic DNAs. Bates et al. and Ji et al. used this approach to impart resistance (i.e., considerably reduced viral titers that abolished or significantly reduced disease symptoms) against Bean yellow dwarf virus (BeYDV) and Beet severe curly top virus (BSCTV), respectively, in Nicotiana benthamiana [6,7]. Ali et al. showed that CRISPR/Cas9 technology could impart molecular immunity against three geminiviruses [i.e., Tomato yellow leaf curl virus (TYLCV), Beet curly top virus (BCTV), and Merremia mosaic virus (MeMV)] in N. benthamiana plants [8], and revealed that a sgRNA designed to target a conserved sequence (TAATATTAC) in the viral intergenic region could be used to target multiple geminiviruses simultaneously [8]. This sequence is conserved among geminiviruses and is also a hallmark of the betasatellites of begomoviruses [3]. Thus, this approach could effectively impart resistance to multiple viruses under natural conditions, where mixed infections predominate (Figure 1).

Efforts to engineer molecular interference against geminiviruses using CRISPR/Cas9 technology require researchers to determine the efficacy of interference and of the sgRNA in directing Cas9 to the viral genome for degradation. One useful strategy is to test the system by transient transformation in N. benthamiana. For example, Ji et al. used transient transformation to correlate Cas9 expression with levels of virus suppression, revealing the importance of using a transgenic line with optimum Cas9 and sgRNA expression [7]. Sufficient levels of CRISPR/Cas9 molecules in plants may be needed to interfere with the invading virus efficiently. Ali et al. tested the efficacy of newly designed sgRNAs in targeting single or multiple viral genomes in systemic N. benthamiana leaves (i.e., leaves that emerge after inoculation) using tobacco rattle virus (TRV)-mediated delivery of sgRNAs [9]. This transient transformation system also enabled an analysis of CRISPR/Cas9 interference activity against the viral sequence in systemic leaves [9].

Although these three studies demonstrated the efficacy of the CRISPR/Cas9 system against geminiviruses in N. benthamiana, transformation in crop plants and field trials are needed to be
Figure 1. Engineering Plant Resistance against Viral Pathogens via the CRISPR/Cas9 System. (A) Graphical representation of a geminivirus with multiple overlapping genes and a conserved nonanucleotide sequence within a hairpin loop in the intergenic region (IR), architecture of molecular components for CRISPR/Cas9 with guide RNAs (gRNAs) against single (upper cassette) or multiple (lower cassette) virus targets. Stable transformation of the CRISPR/Cas9 system in plants. Transgenic plants expressing CRISPR/Cas9 are challenged with virus agroinfiltration (red syringe) or under natural conditions to determine the efficacy of resistance. The CRISPR/Cas9 transgenic plants (T) show virus resistance upon agroinoculation, while non-transgenic control plants (C) develop typical disease symptoms, indicating susceptibility. (B) Stepwise depiction of CRISPR/Cas9-mediated virus interference in the plant cell nucleus. (1) Components of the CRISPR/Cas9 machinery, gRNA and Cas9, are expressed from the plant genome. (2) Assembly of the gRNA–Cas9 complex. (3) Upon virus challenge, the geminivirus infects the plant cell. (4) The single-stranded DNA (ssDNA) geminivirus genome replicates through the double-stranded DNA (dsDNA) replicative form followed by rolling circle replication (RCR) to produce multiple viral copies. (5) The gRNA–Cas9 complex targets the viral dsDNA at complementary target sites and (6) cleaves the viral genome via double-strand break (DSB) formation, which can be repaired by non-homologous end joining (NHEJ) repair. Alternatively, the formation of DSBs can lead to degradation of the viral genome.

conducted to evaluate CRISPR/Cas9-mediated resistance in natural conditions. One important question is whether targeted cleavage of a geminivirus could accelerate the evolution of viral strains that can evade the Cas9 machinery, perhaps by mutagenesis of the target or PAM sequence. The rate at which the virus evolves to evade the CRISPR/Cas9 machinery also remains to be determined. One major concern for CRISPR/Cas9 approaches is the potential for off-target effects on the plant genome. The CRISPR/Cas9 system might target other genomic loci and introduce mutations that could
affect crop productivity; constitutive expression of the CRISPR/Cas9 system can exacerbate this problem by presenting more opportunities to alter the genome. However, off-target Cas9 activities in the plant genome have been detected only rarely [9]. These activities can be further minimized by engineering transient Cas9 expression that is activated by viral infection or chemical induction [10]. Also, Baltes et al. showed that one sgRNA targeting the BeYDV genome could confer plant resistance without inducing cleavage; this suggests that catalytically inactive Cas9 (dCas9) can be used to mediate virus interference and thereby eliminate concerns of off-target activities in the plant genome [6]. Thus, expression of a dCas9 variant might interfere with viral replication while reducing the potential for off-target effects. However, this remains to be tested. Moreover, recent work has identified Cas9 enzymes that give fewer off-target effects, thus further reducing this concern in plants [11]. However, additional research is required to test whether the platform can be adapted to target RNA viruses using other Cas9 variants [12].

Harnessing the CRISPR/Cas9 machinery to engineer plant resistance to viral pathogens also opens the possibility of addressing basic questions in virus infection and plant host resistance. For example, the CRISPR/Cas9 platform could be used to investigate the evolution of the viral genome to counteract plant immunity, by examining the genomes of viruses that escape recognition by the CRISPR/Cas9 system. The CRISPR/Cas9 platform could also be used for targeted mutagenesis to identify host factors that control plant resistance and susceptibility to viral infection. Thus, CRISPR/Cas9 technology offers a promising approach for understanding and engineering resistance to single and multiple viral infections in plants.

Trends in Plant Science

Glossary

Amplitude: the height difference between the peak and the trough of a wave.

Circadian: literally ‘about a day’ (~24 h).

Clock: generally refers to the entire circadian system, but can be used to refer specifically for the oscillator.

Input: the pathways via which information from the environment, such as changes in light and temperature, are transduced to the oscillator.

Oscillator: the cell-autonomous timekeeper responsible for generating self-sustained rhythmicity.

Output: the pathways linking the oscillator with the diverse biological processes it controls.

Period: the time taken for one complete cycle.

Phase: the relationship of a point in a rhythm to a marker such as another rhythm, for example the relationship of peak expression of a gene to daybreak during a day-night cycle.

Photoperiod: day length; the duration of daylight every 24 h.

All plants live with daily oscillations in their environment and have developed endogenous circadian (see Glossary) systems that allow them both to anticipate these changes and to respond appropriately. A simplified, although workable, view of the circadian system is that it consists of three parts; the oscillator that generates rhythmicity, the input pathways by which the oscillator is set (entrained) to the environment, and output rhythms which are regulated by the oscillator. The oscillator mechanism has been largely studied in Arabidopsis and is based on a series of interlocking transcriptional–translational feedback loops of core oscillator components (Figure 1) [1]. All higher plants that have so far been examined have orthologs of most of the Arabidopsis oscillator genes and the fundamental mechanism of the oscillator appears to be widely conserved [2]. Although by definition circadian rhythms have ~24 h periods, exact period lengths can vary by a few hours and the amplitude and phase may also differ.

The circadian oscillator controls a diverse range of processes including daily fluctuations in photosynthetic capacity, resistance to abiotic and biotic stress, flower opening, scent production, and the expression of a large percentage of the

Spotlight

The Impact of Domestication on the Circadian Clock

Ekaterina Shor1 and Rachel M. Green1,.*2

A recent publication shows that, during their domestication and spread from Equatorial South America, circadian rhythms of tomatoes have been modified. The modifications have resulted in tomato plants that are adapted to growing under the long day conditions characteristic of summers at higher latitudes.

References