



Use of CRISPR/CAS9 System in Engineering Plant Resistant to Gemini Virus, an Emerging Threat

Pakeeza Abid ^a, Muhammad Babar Malook ^b,
Sidra Adil Khan ^c, Muhammad Riaz ^d,
Hassan Rehman Ali ^b and Kashif Kareem ^{e*}

^a Sargodha Medical College, University of Sargodha, Pakistan.

^b Department of Plant Pathology, Institute of Plant Protection, Muhammad Nawaz Sharief University of Agriculture Multan, Pakistan.

^c Department of MBA, IQRA University, Karachi, Pakistan.

^d Department of Plant Pathology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan.

^e Department of Biotechnology, COMSATS University Islamabad, Abbottabad Campus, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Gemini viruses account for destructive scourge wastes that endanger nourishment safety. The genomic sequence of Gemini virus comprises of a round or disk shaped, unique or isolated stranded or grounded DNA crumb, which set off a duplex DNA replica center in the parenchyma cell core and translate almost seven to four universal polypeptide Weed attribute manipulate needs effectual pick out genetic editing machinery. Clustered regularly interspaced palindromic repeats accompanying (CAS) type II organization cast off select genomic changing implementation beyond the organisms that have nucleus in their cell containing plants that cause resistance of these devastating Gemi virus. We know the evolution (TRV) tobacco rattle virus, which is, mediated

*Corresponding author: Email: kashifkarim68@gmail.com;

during the genomic changing or during editing in tobacco plant family *not long ago*. We know that tobacco rattle virus also affects the new and newly growing plantlets that have possess a very small size genomic size that help in multiplication and binary fusion and also in cloning and agricultural agro infections that cause the plants to diseases. closer, constant action and distinctiveness of the tobacco rattle virus, which is mediated CRISPR Cas9 system for targeted modification of the tobacco rattle family plant *Nicotiana benthamiana* genome. Informational and experimental report perseverance TRV-moderate Cas-9 task for about 30 days agroinfection. In addition, our results show or demonstrate that TRV make up genomic sequence changing illustrated no in-exact work generable in exact arguing the exactness of the organization for vascular plantlets genomic sequencing scheme. Are hold of at once, the particular statics authorize reasonableness arousing chances of utilized the viral particle as a make peace CRISPER/Cas9 for chosen manipulate of the plant genomic sequence.

Keywords: *Palindromic; multiplexing; CRISPR Cas9; post-agroinfection; DNA replication.*

1. INTRODUCTION

Gemini viruses, the matching that equilateral or triangle molecules, affect the crops that contain the edible food that we ate and also affects the crops of both seasons, affects the plants that are grown in garden for the decorative purposes and also plants in different types of forests and also affects the plants that provides sustainable food resources to human being losses are caused by them around the world [1]. The occurrence of these types of disease increased with the use of pesticides and as the insect's produced resistance against them in the last 20 years. The disease caused by this virus has enormous impact in different areas of agriculture like in cotton maize and wheat because it cause the curling of leaf and the plants unable to produce the crops or yield due to this virus in Africa and Asia3–5 [2]. This virus also cause disease in tomato plants that show the symptoms or signs of curling of leaf and stunt growth contribute the major source of disease in the tomato plants worldwide. The virus causes these disease are called the Gemini virus having the property that have very small genomic DNA and also possess the coding capacity that interact with the host plants and cause them disease because it has a strong host cellular machinery to infect the plants [3].

For the replication of chromosomal DNA of plants and viral DNA of plants, Gemini virus has the ability to reprogram their cell cycle of the cell infected with it. The Gemini virus enters into the host plants cells and perform many function such as the virus change the expression of gene in the host cell of plants, may cause the blocking the host plants cell hormones trafficking all across the plants, it may also cease the normal hormones delivery in plants cell and cause the cell death of plants, may reduce the plants

hormones turnovers and affects the signaling pathway of the plants that are very serious condition of the plants. Plants become wilt and ultimately death occur [4].

Gemini virus has multiple type of suppressors like silencing suppressors that has the ability to interfere with the plants cell production of the small interfering RNA (siRNA), also has the ability to change the DNA methylation, that has the ability to produced many types of abnormalities in plants cells. It also has the ability to change the Micro RNA, which also produced the many types of the abnormalities in plants cells [5]. Gemini virus mostly exist in the form of colony or complexes, and all these complexes or colony may infect the plant cell and cause the death of plants in most cases. Each individual of the Gemini virus has unique characteristics, each colony or individual virus undergo genome multiplication, mutation and the new strains of the virus produced after recombination and assortment.

In particular, whiteflies excess use of pesticides and insecticides over the crops for the elimination of the whiteflies and many types of the pathogens like bacterial, viral and fungal they may produce resistance in the pathogens, and these pathogens may affects the crops more when spread in new region. The Gemini virus are more resistant, produced infection in plants crops when combined with other virus, and cause the diseases [6]. Gemini virus undergo mutation or change its strain and produced more complexes, than this virus spread to new environment and enter into the new hosts due to environmental changes or the human activity and impose threat to the plants especially edible plants and cause the food and security threats in the world. Gemini viruses belong to a family Geminiviridae, with single stranded ssDNA of 2.7-2.8 kb base

pair having the single circular genome. The virus mainly transmitted from insect to plants and blocked their signaling pathway and curling of leaf occur, plants become welts and death occur of the plants [7].

Gemini virus has 2 main components as like named as DNA A and other is the DNA B. both of these components play an important role in the production of virus in the host cell of plants and the proliferation of the virus colony in the host cell. As we know Gemini virus has two components, and these components contain regions known as motifs. These motifs play an important role in the expression of the genomic DNA in the host cell and vital for the replication of the DNA. These motifs form a structure and this structure known as a stem loop- structure that has multiple types of conserved nucleotide TAAATATTAC. These conserved regions play vital role in the initiation of replication [8].

Sometimes the Gemini virus B components produces mutate or defective DNA that can also function as a defective interfering DNA. This defective interfering DNA function as to produce the disease or infection but that infection have low severity of the disease when this defective Interfering DNA compare with the whole genomic DNA of the Gemini virus cellular components [5]. Some of efforts done in engineering the Gemini virus resistance in crops and some strategies are the viral protein mediated resistance, viral RNA mediated resistance, host derived resistance and also Non- viral mediated protein resistance. Scientist continuing effort to engineering this type of Gemini virus that have all above the properties [9]. Nevertheless, the Gemini virus evolved from the viral protein mediated resistance, viral RNA mediated resistance, and host derived resistance and Non- viral mediated protein resistance strategies have some disadvantages like the mutation of the virus; fast recombination may also cause the mutation in the genome of the virus. Therefore, scientist left these techniques for the formation of Gemini virus while using these techniques. They use another techniques name as substantial resistance techniques for the formation of Gemini virus, and this technique is more effective than the others [10].

Substantial resistance techniques need the change or editing of the genomic DNA of the Gemini virus. Therefore, for editing the genomic DNA of the virus a techniques known as Clustered Regularly interspaced short palindromic repeat is used. Its small sequence of

RNA that found in bacteria and in archaea. CRISPRs combined or associated with Cas9 that has a potential used for the genomic editing of the Gemini virus. The techniques Clustered regularly interspaced short palindromic repeat (CRISPRs) with cas9 used for editing all types of the virus genome. Its small pieces of DNA called spacers [11].

During any type of viral infections, clustered regularly interspaced short palindromic repeat (CRISPRs) use a small pieces of RNA to direct the cas9 enzyme to the similar sequences. A special protein found in it known as a "spacer" that function to transcribe into the smaller RNA. CRISPRs guides the system to matching the similar DNA while the Cas9 function as bind and cut the DNA, similar function as shutting of the targets gene off [12]. For the modification of the targeted DNA of the Gemini virus, so that it may not infect the plant cell further, an RNA known as single guide RNA used with the cas9 to the cleavage of the targeted DNA sequences in both the eukaryotic cell and the prokaryotic cell. SgRNA combined with the cas9, perform endonuclease activity, and cleave the targeted DNA of the virus [13].

Clustered regularly interspaced short palindromic repeat (CRISPRs) cas9 application give us off targets genome that are non-specific and engineered through these techniques. This techniques has also potential for the off targets effects on plants genomic sequences especially during the Gemini virus and this review highlights either our CRISPR/CAS 9 system exhibit these off target activities or not [14].

Studied shows that the Clustered regularly interspaced short palindromic repeat (CRISPRs) cas9 techniques play an important role in controlling the spread of disease through the Gemini virus. When the virus genome editing done than it could not have negative effect on the plants. Therefore, when the Gemini virus genome editing done while targeting the viral genomic DNA, its infection rate would be lessened or harnessed than previously noted. This CRISPRs cas9 also show positive effects while producing the immunity in plants especially molecular immunity in plants like tobacco plants. sgRNA designed to targets a sequence known as a conserved sequences for the in the virions intergenic virions to targets the multiple Gemini virus simultaneously [15]. In *Nicotiana Benthamiana*, carry gRNA that has function as a modification and targets it. Tobacco rattle virus ability to produce or developed a vector that

function the modification in the *Nicotiana Benthamiana* [16].

2. METHODOLOGY

Tobacco rattle virus is a plant pathogenic virus. It mainly affects the ornamental plants and cause the infections in plantlets. Tobacco rattle virus also has the ability to use as a vector. TRV use as a vectors in a bipartite, it means that is has two RNA. One is the RNA1 and other is the RNA2 genomic sequences of TRV. RNA1 function as a virus-induced gene silencing (VIGS), its major function in the genomic studies in the various plants especially in the species of tobacco rattle virus genome studies. RNA2 has function as a posttranslational modification of the tobacco rattle virus genome, means its function in the exogenic gene fragments modification [17]. Tobacco rattle virus TRV have function in delivering the gRNA molecules, modified the growing tissues. As we know that TRV virus are so small with length of about 2-3 kb and cannot delivered with the cas9 molecules into the plants. Therefore, that why it delivered with the agro infiltration that reconstituted in the leaves of *Benthamiana* than finally expressing in the cas9 [18].

During experimental analysis, RNA1 genome combined with the RNA 2 vector and form a culture known as a mixed agrobacterium culture. The PDS gene was driven from the PEBV promoter. The experimental analysis show that gRNA bind with the PDS gene. A negative control also used in an experiment that was a clone of gRNA empty vector. For assessing the PDS target sequences, sample were obtained after 7, 15 and 30 days of post infiltration with some specific condition like 3 plants with PCR done amplifying 797 base pair fragments of flanking he target site. The final PCR product were analyzing or assay [19].

3. RESULTS AND ANALYSIS

After assaying the product, the results assessed. The results show that after 7, 15 and 30 days of post infiltration with specific PCR condition the sample done into the machine or software and it give us the modified efficiency of the product. Tobacco rattle virus that mediated with Clustered regularly interspaced short palindromic repeat (CRISPRs) cas9 editing indicate the activity of the system. This activity important for the recovery of the mutant seed progeny and for the modification of the of the germline cells [20,21].

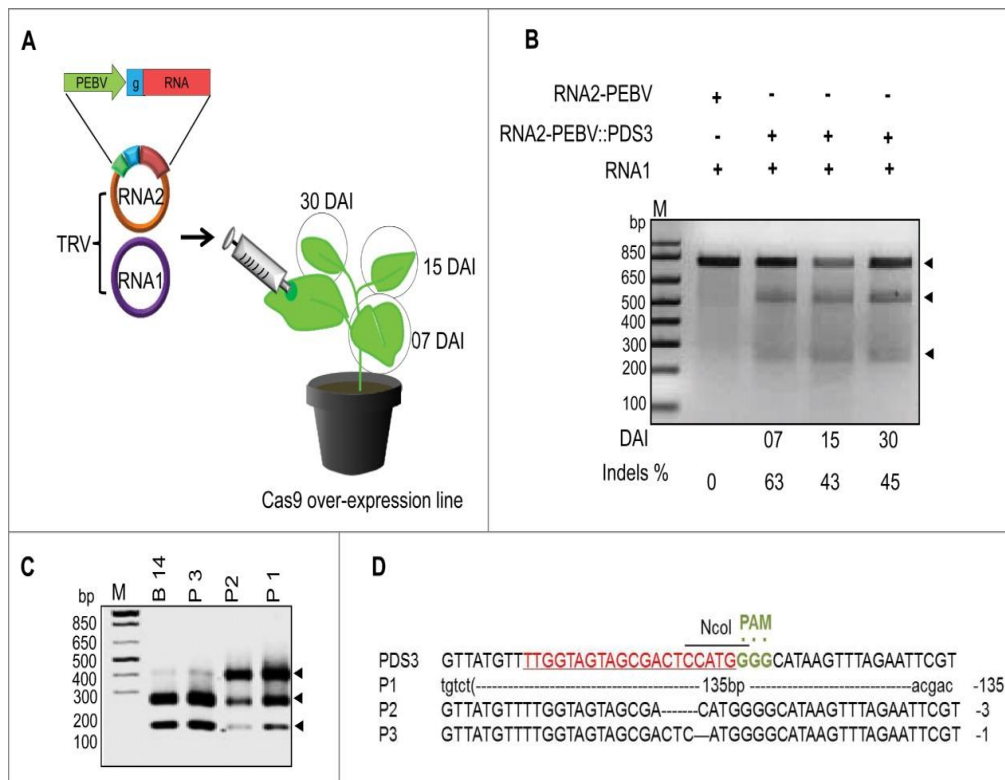


Fig. 1.PDS3 gene thatmediated by CRISPRs cas9 in persistence tobacco rattle virus TRV

As we know that tobacco rattle virus TRV infects all types and all parts of plants especially meristematic tissues and plantlets, here we tested with experiment whether the plant progeny infected with the pRNA2. PDS gRNA have done some modification in genomic in the targeted PDS gene [22]. To check the levels of infiltration leaves, three matured seed grouped in one pool. The experimental analysis performed. Leaves taken from the plants in a tube, experimental analysis were performed with the using of protocol and total of four pools containing the WT control [23]. PCR and some other protocol also performed to analyze the final product. PCR is done for amplification of the product. To access the targeted sequence modification in these pools a restriction enzyme is used known as NcoI restriction digestion. The 404 complementary base pair of the DNA fragments delivered to the restriction digestion enzyme.

An NcoI band enzyme resistance band only developed in that pool that are infect with PRNA2, PEVB. PDS compared with the WT control. It shows that the presence of genomic modification in seed progeny [24]. After the

amplification, the PCR product formed. These PCR products cloned in a vector and that cloned results delivered for sequencing known as Sanger sequencing. This Sanger sequencing tells us about the presence of modification of in the target site [25]. The sequencing data provide the evidence about recovering these plants, about bringing the target modification, efficiency and about the need of transformation and tissue culture [26].

The detection of tobacco rattle virus TRV in early transmission indicate that the infection is persistence and there is a need of improvements to recover the plants [27,28]. The results indicate that more improvements are required of germinal transmission and the recovery of mutant plants from the seed progeny [29]. It is fair to say whether the germinal transmission is off target activity or not [30,31]. *N. benthamiana* genome were screen for perfect and imperfect matches. The twenty-nucleotide sequences gRNA identified the candidate for the editing of the target genome and thirteen delivered to T7EI for restriction protection assay [32].

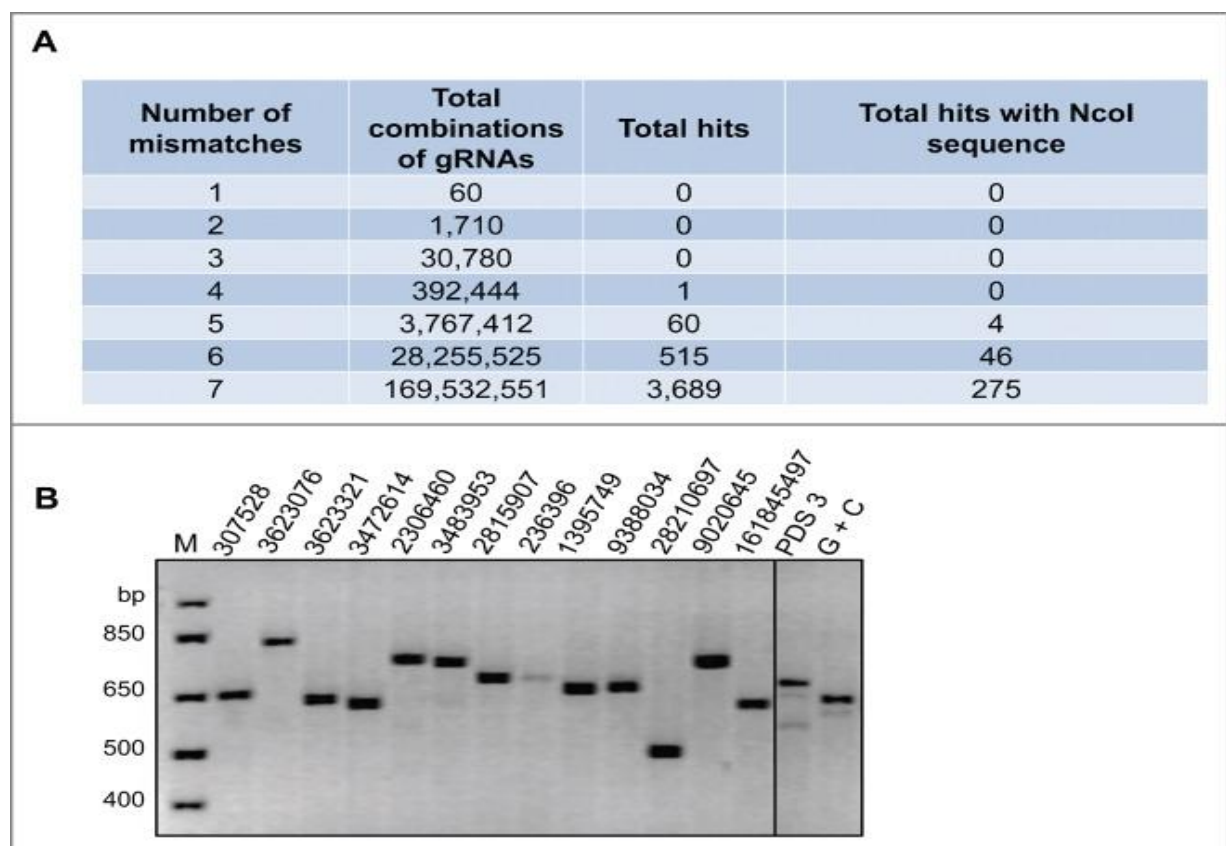


Fig. 2. TRV-based CRISPR/Cas9 system exhibited no apparent off-target effects in *N. benthamiana* genome

4. DISCUSSION

Nevertheless, off beam Cas9 occupation in the vegetation genomic and HapMap disclosed hardly [33]. the previously mentioned, interest supplementary diminished manipulate short lived Cas9 articulation which is operated by microorganism or impel [34]. SgRNA chosen the TRV epigenome advise, immunity enantiomer move dividing,; advises that catalyzer immobile Cas9 (dCas9) can brought to bear interpose viral involvement and whereby discard interests of beam occupation in the vegetation plant genomic sequence [35]. Moreover, fresh slog has recognized Cas9 stimulant that offer few off center influence , additionally weaken interest in vegetative plantlets [36]. However, further experiment is essential to examine either the objectives to adjust chosen more Cas9 variation [37].

5. CONCLUSION

In deduction, own assignment drives endurance of TRV- moderate CRISPR/Cas9 modifying and the likelihood of enhance this techniques to convert offspring bring the pick out the changing whereby depart from the demand from the explant culture and refashioned transformation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Zaidi SS-e-A, Mansoor S, Ali Z, Tashkandi M, Mahfouz MM. Engineering plants for geminivirus resistance with CRISPR/Cas9 system. *Trends in plant science*. 2016;21(4):279-81.
2. Ali Z, Ali S, Tashkandi M, Zaidi SS-e-A, Mahfouz MM. CRISPR/Cas9-mediated immunity to geminiviruses: differential interference and evasion. *Scientific reports*. 2016;6(1):1-13.
3. Raja P, Sanville BC, Buchmann RC, Bisaro DM. Viral genome methylation as an epigenetic defense against geminiviruses. *Journal of virology*. 2008;82(18):8997-9007.
4. Sahu PP, Prasad M. Application of molecular antiviral compounds: novel approach for durable resistance against geminiviruses. *Molecular biology reports*. 2015;42(7):1157-62.
5. Morilla G, Castillo AG, Preiss W, Jeske H, Bejarano ER. A versatile transreplication-based system to identify cellular proteins involved in geminivirus replication. *Journal of virology*. 2006;80(7):3624-33.
6. Nagar S, Pedersen TJ, Carrick KM, Hanley-Bowdoin L, Robertson D. A geminivirus induces expression of a host DNA synthesis protein in terminally differentiated plant cells. *The Plant Cell*. 1995;7(6):705-19.
7. Nash TE, Dallas MB, Reyes MI, Buhrman GK, Ascencio-Ibañez JT, Hanley-Bowdoin L. Functional analysis of a novel motif conserved across geminivirus Rep proteins. *Journal of virology*. 2011;85(3):1182-92.
8. Nawaz-ul-Rehman MS, Fauquet CM. Evolution of geminiviruses and their satellites. *FEBS letters*. 2009;583(12):1825-32.
9. Mansoor S, Zafar Y, Briddon RW. Geminivirus disease complexes: the threat is spreading. *Trends in plant science*. 2006;11(5):209-12.
10. Mansoor S, Briddon RW, Zafar Y, Stanley J. Geminivirus disease complexes: an emerging threat. *Trends in plant science*. 2003;8(3):128-34.
11. Legg JP, Fauquet CM. Cassava mosaic geminiviruses in Africa. *Plant molecular biology*. 2004;56(4):585-99.
12. Kong L-J, Hanley-Bowdoin L. A geminivirus replication protein interacts with a protein kinase and a motor protein that display different expression patterns during plant development and infection. *The Plant Cell*. 2002;14(8):1817-32.
13. Kaliappan K, Choudhury NR, Suyal G, Mukherjee SK. A novel role for RAD54: this host protein modulates geminiviral DNA replication. *The FASEB Journal*. 2012;26(3):1142-60.
14. Hanley-Bowdoin L, Bejarano ER, Robertson D, Mansoor S. Geminiviruses: masters at redirecting and reprogramming plant processes. *Nature Reviews Microbiology*. 2013;11(11):777-88.
15. Gutierrez C. DNA replication and cell cycle in plants: learning from geminiviruses. *The EMBO journal*. 2000;19(5):792-9.
16. Zaidi SS-e-A, Tashkandi M, Mansoor S, Mahfouz MM. Engineering plant immunity: using CRISPR/Cas9 to generate virus

- resistance. *Frontiers in plant science*. 2016;7:1673.
17. Fondong VN. Geminivirus protein structure and function. *Molecular plant pathology*. 2013;14(6):635-49.
 18. Briddon R, Pinner M, Stanley J, Markham P. Geminivirus coat protein gene replacement alters insect specificity. *Virology*. 1990;177(1):85-94.
 19. Baltes NJ, Hummel AW, Konecna E, Cegan R, Bruns AN, Bisaro DM, et al. Conferring resistance to geminiviruses with the CRISPR–Cas prokaryotic immune system. *Nature Plants*. 2015;1(10):1-4.
 20. Navas-Castillo J, Fiallo-Olivé E, Sánchez-Campos S. Emerging virus diseases transmitted by whiteflies. *Annual review of phytopathology*. 2011;49:219-48.
 21. Gonzales-Salazar R, Cecere B, Ruocco M, Rao R, Corrado G. A comparison between constitutive and inducible transgenic expression of the PhRIP I gene for broad-spectrum resistance against phytopathogens in potato. *Biotechnology letters*. 2017;39(7):1049-58.
 22. Wright AV, Nuñez JK, Doudna JA. Biology and applications of CRISPR systems: harnessing nature's toolbox for genome engineering. *Cell*. 2016;164(1-2):29-44.
 23. Schaeffer SM, Nakata PA. CRISPR/Cas9-mediated genome editing and gene replacement in plants: transitioning from lab to field. *Plant Science*. 2015;240:130-42.
 24. Ji X, Zhang H, Zhang Y, Wang Y, Gao C. Establishing a CRISPR–Cas-like immune system conferring DNA virus resistance in plants. *Nature Plants*. 2015;1(10):1-4.
 25. Ali Z, Abul-Faraj A, Li L, Ghosh N, Piatek M, Mahjoub A, et al. Efficient virus-mediated genome editing in plants using the CRISPR/Cas9 system. *Mol Plant*. 2015;8(8):1288-91.
 26. Merchante C, Brumos J, Yun J, Hu Q, Spencer KR, Enríquez P, et al. Gene-specific translation regulation mediated by the hormone-signaling molecule EIN2. *Cell*. 2015;163(3):684-97.
 27. Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. *Science*. 2016;351(6268):84-8.
 28. O'Connell MR, Oakes BL, Sternberg SH, East-Seletsky A, Kaplan M, Doudna JA. Programmable RNA recognition and cleavage by CRISPR/Cas9. *Nature*. 2014;516(7530):263-6.
 29. Abouzid A, Hiebert E, Strandberg J. Cloning, identification, and partial sequencing of the genomic components of a geminivirus infecting the Brassicaceae. *Phytopathology*. 1992;82(1070):2320-5.
 30. Murrant A, Mayo M. Satellites of plant viruses. *Annual Review of Phytopathology*. 1982;20(1):49-68.
 31. Fauquet CM, Bisaro D, Briddon R, Brown J, Harrison B, Rybicki E, et al. Revision of taxonomic criteria for species demarcation in the family Geminiviridae, and an updated list of begomovirus species. *Archives of virology*. 2003;148(2):405-20.
 32. Thresh J, Otim-Nape G, Thankappan M, Muniyappa V. The mosaic diseases of cassava in Africa and India caused by whitefly-borne geminiviruses. *Review of Plant Pathology*. 1998;77(9):935-45.
 33. Czosnek H, Laterrot H. A worldwide survey of tomato yellow leaf curl viruses. *Archives of virology*. 1997;142(7):1391-406.
 34. Rojas MR, Noueiry AO, Lucas WJ, Gilbertson RL. Bean dwarf mosaic geminivirus movement proteins recognize DNA in a form-and size-specific manner. *Cell*. 1998;95(1):105-13.
 35. Briddon R, Mansoor S, Bedford I, Pinner M, Saunders K, Stanley J, et al. Identification of DNA components required for induction of cotton leaf curl disease. *Virology*. 2001;285(2):234-43.
 36. Briddon RW, Bull SE, Amin I, Idris AM, Mansoor S, Bedford ID, et al. Diversity of DNA β , a satellite molecule associated with some monopartite begomoviruses. *Virology*. 2003;312(1):106-21.
 37. Eagle PA, Orozco BM, Hanley-Bowdoin L. A DNA sequence required for geminivirus replication also mediates transcriptional regulation. *The Plant Cell*. 1994;6(8):1157-70.

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