антиоксидантные ферменты. Эти данные показывают, что салициловая кислота является важной сигнальной молекулой, которая способна регулировать системную резистентность и обладает большим потенциалом для растений с повышенной устойчивостью к патогенным инфекциям.

Список использованной литературы:

1. Аббинк, Т.Е., Пирт, Дж.Р., Мос, Т.Н., Баулкомб, Д.К., Бол, Дж.Ф. и Линторст, Х.Дж. (2002) «Замалчивание гена, кодирующего белковый компонент кислород-выделяющего комплекса фотосистемы II, усиливает репликацию вируса в растениях» Вирусология, 295, 307–319.
2. Апель, К. и Хирт, Х. (2004) «Активные формы кислорода: метаболизм, окислительный стресс и передача сигнала» 373–399.
3. Бакстер, А., Миттлер, Р. и Судзуки, Н. (2014) «АФК как ключевые игроки в передаче сигналов стресса у растений». Журнал экспериментальной ботаники, 65, 1229–1240.
4. Дас, К. и Ройчоудхури, А. (2014) «Активные формы кислорода (АФК) и реакция антиоксидантов как поглотителей АФК во время экологического стресса у растений». 53.

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CONSTRUCTION OF PLASMID VECTOR PLATFORM FOR EXPRESSION OF CAS13 PROTEINS IN *NICOTIANA BENTHAMIANA* PLANTS

Dospambetov N., Iksat N., Omarov R.

n9708@bk.ru

Student of Faculty of Natural Sciences at L.N. Gumilyov Eurasian National University,
Nur-Sultan, Kazakhstan
Scientific Supervisor-Nurgul Iksat

Introduction.

Recently discovered technology of CRISPR/Cas13 system has revolutionized application of RNA. Namely, its implementation in various plant species has already demonstrated the advantage of CRISPR over traditional methods of RNA interference. Despite the advancements in pesticide technology, each year farmers lose a significant number of crops due to plant virus infections. With the rise of global population demands for food are rising each year. Thus, there is an urgent need for an effective method of minimizing the effect of plant viruses on crop yield. CRISPR/Cas13 system has already been tested in some plant species and it demonstrated itself as an reliable and efficient method of developing plant immunity for viruses. Although Cas13 is a rapidly developing tool in RNA biotechnology, there is a distinct lack of research in plasmid vector construction.

Li (2020) with his colleagues in Zheijang university, noted the drawbacks of transgene-mediated CRISPR-Cas delivery, which includes unexpected alterations as well as damages in the genome. To attain high frequency mutations Li engineered a vector based on plant negative-strand RNS to DNA-free CRISPR/Cas9 delivery. To make sure that there were no off-target mutations, 13 potential sites in *N. benthamiana* genome were subjected to an amplification study for possible mismatches. The result of the study demonstrated no off-target mutations. Using rhabdovirus NYSV as a vector allowed Li to circumvent stages of isolating plant tissues and injecting CRISPR-Cas9 into intact plants instead. This vector also demonstrated amazing stability, making it possible to deploy mechanical passage without using Agrobacterium pathogens [1].

Since the late 1980s all the attention of the virology field has been focusing on the implementation of viruses as vectors for protein overexpression. Cody and Schothof (2019) contemplate on several major recent advances in viral vector technology. Namely, amplifying

gene expression levels using viral gene delivery techniques, RNA silencing methods using viral vectors and finally viral vector-based portage of gene editing machinery. Sequencing of viral genomes significantly uncomplicated upcoming functional genetic techniques. They also noted that the deployment of translational viral RNA enhancers become vital in protein overexpression as much as the use of DNA promoters are. Also, gene-replacement and gene-insertion techniques emerged as solutions to go around the plant transformation step. Additionally, Cody and Schothof report on the expansion of viral vector application outside of virology. The discovery of sequencing technologies combined with RNA silencing has given accumulative effects on plant functional genomics [2].

Puchta (2018) mentions that for a long amount of time plant breeding was based on very imperfect and non-specific technologies. But the situation changed dramatically with the discovery of sequence specific nucleases (SSN). SSN based double strand break became a very important milestone in the development of genome technologies. Due to the limited number of target sites several synthetic nuclease technologies were engineered, namely transcription activator-like effector nuclease (TALENs) and zinc-finger nuclease. Though none of these methods demonstrated as much flexibility as the CRISPR/Cas9 system, which allowed induction of double strand breaks at any site in the genome. Protein coding sequences became the main object of plant breeding, while regulatory sequences were largely ignored [3].

Bao et al. (2019) noted that the CRISPR/Cas9 system has turned into the ultimate gene editing tool thanks to its advantages over TALEN and ZFN. The main one being that it does not require a protein engineering process. While mentioning the success of the CRISPR system, Bao et al. also mentions the need of additional research to optimize and analyze for possible off-target mutations using bioinformatics tools (namely CRISPR-P 2.0 and CRISPR-GE). One of the pressing issues for mutant screening remains the link between the target genes and visible plant phenotype. Bao et al. highlights that one of the most crucial applications of CRISPR/Cas9 is increasing crop varieties, the importance of which is being elevated each year due to global population explosion. This could be achieved by modifying gene that are responsible for flowering, quality improvement and stress tolerance [4].

Stivashev et al. (2021) highlighted the advantages of using Cas9-gRNA system over TALEN and ZFNs, which is the lack of need for protein engineering. The study of the Cas9 system had been mostly focused on NHEJ, a reliable method of gene knockout. Though the application of gene knockout remains restricted, which underlines the deployment of HDR. While giving more opportunities regarding gene editing, HDR is more challenging and complicated to achieve. Stivashev et al. gave a novel report on direct delivery mediated acquisition of mutagenesis using Cas-gRNA system. During their experiment 12 different sites were selected for cleavage to examine the ability of RNA guide Cas9 to generate DBSs. DNA vectors with meganucleases were deployed to compare the performance of Cas-gRNAs. As expected, Cas9 demonstrated a considerably higher frequency of mutation than in the control group. One interesting feature that was observed in the experiment is that Cas9-gRNAs mostly induce single nucleotide insertions and deletions, while the size of the mutations induced by meganucleases varied. Stivashev et al. (2021) noticed that integration of Cas9 expression cassettes can cause somatic mutations, which was evident from the lack of proper Mendelian segregation. Utilising the experiment with bombardment with fluorescent marker MoPAT Stivashev et al. (2021) demonstrated that Cas9-gRNA can cause mutations at several loci at once.

Stivashev et al. also mention that some plant species (*Arabidopsis*, wheat) induced with Cas9-gRNA do not follow Mendelian segregation. This was followed by an experiment that confirmed that delivery of Cas9-gRNA using a DNA vector is 100 times more likely to cause somatic mutation than using homing endonucleases. Overall, Cas9-gRNA delivery yielded high frequency of mutagenesis and lower risks of off-target mutations. Stivashev et al. states that this might elevate the use of targeted mutagenesis in to produce new allele variants [5].

Recently a new type of Cas13 proteins called Cas13a were discovered. Due to the lack of data on molecular cues on their nuclease activity to use them as tools, there is a need for a closer

look. In order to investigate distinct activities of A-cleaving Cas13a subfamily Doudna et al. (2017) made a crystal structure using single wavelength anomalous dispersion (SAD). Thus creating an opportunity to compare it with the U-cleaving subfamily members that were described previously. The structure model of Cas13a demonstrated that its secondary structure mostly consists of six α-helix domains. Doudna et al. also revealed the details of spacer recognition and activation of Cas13a crRNA has a preference due to a set loop confirmations and a number of 5' handle-Cas13 contacts. Overall, the experiment demonstrated the molecular mechanism of catalytic activities of the newly found Cas13a enzyme as well as its application in diagnostics [6].

As Chuang (2020) highlighted, CRISPR/Cas has a huge potential in the application for gene therapy and crop development though it also has some serious limitations and drawbacks worth considering. Being derived from bacteria, there is a danger of the host immune response being triggered. Out of all the types of CRISPR/Cas system Cas9 system is the most widely utilized one up to now. This is due to its flexibility and relative simplicity of use. It belongs to type 2 CRISPR/Cas system, meaning it contain only one effector nuclease. The endonuclease property of CRISPR/Cas has not only been proven to be useful in modifying DNA but RNA as well. Several strategies such as gene disruption, precise repair, and targeted insertion can be used to obtain the desired modification. The application of Cas system further expanded with the discovery of RNA targeting Cas13 family. Just like DNA-editing Cas9, Cas13s efficiency and specificity turned out to be much higher than other RNA-editing methods. Another interesting approach is to use non-catalytic Cas9 (or dead Cas9) protein to obtain transcriptional repression, meaning dCas will be fused with gene activator to prevent transcription of the given gene. This method is important because it can be deployed in base-repair, which constitutes the majority of the mutations happening in organisms.

There are several modes of delivery of CRISPR/Cas system: DNA plasmid, RNA, and ribonucleoprotein (RNP) complex. Overall, all three methods are considered effective, though each one have their unique challenges. Delivery methods are separated into two: viral and non-viral vectors. So far in the use of CRISPR/Cas system in vivo viral vectors have proven themselves as more effective than non-viral ones. Delivery systems of CRISPR/Cas have been proven to be effective for both aquaculture animals and plants in many experiments. The delivery in plants can work either by stable or transient approaches [7].

Since viral diseases targeting plants cause huge amounts of economical damage every year, early detection and diagnosis of these diseases are needed. Fast and cheap testing kits for viral diseases are becoming a global necessity. CRISPR/Cas system has proven itself useful not only in gene editing but also in nucleic acid testing. Many viral diseases are emerging every year and treating them is not economically feasible, thus early detection and prevention is a considerably sustainable way of dealing with them. Nucleic acid based kits give very accurate diagnosis many viral diseases. Though this technique mostly relies on real-time reverse transcription PCR, a tool that demands high-cost equipment and trained specialist. Because of impracticality of qRT-PCR, methods such as recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP), and clustered regularly interspaced short palindromic repeats (CRISPR) became good alternatives for NAT tools. Both RPA and LAMP have been deployed to perform detection of viruses such as Newcastle disease virus, influenza, and SARS-Cov2. However, these two methods lack the sensitivity and throughput CRISPR/Cas possess. Usefulness of CRISPR/Cas systems in diagnosis were confirmed with successful deployment of Cas9 for detection of Zika and African swine fever virus, Cas12 for human papillomavirus (HPV), and Cas13 for lymphocytic choriomeningitis virus (LCMV) [8].

Plant breeding has been one of the principals means of countering the economic effect of viral diseases for a long time. However, this method has some major limitations, including the high cost and tedious labour required. Thus, targeting the host-pathogen interaction in order to grant the plants viral resistance is emerging as a promising alternative. The recent discovery of Cas13a let us solve many challenges in RNA editing. Recent experiments conducted by Aman

and his colleagues confirmed that, Cas13a system is able to edit plant viral RNA with high precision. It is revealed that the function of Cas13a is affected by 3' flanking site of the protospacer and secondary structures, which makes the endonuclease activity of Cas13a quite malleable [9].

Zhang et al. (2019) argued that by taking advantage of the fact that eukaryotic viruses are vulnerable to the CRISPR/Cas system, it is possible to build methods to build resistance to various viruses that infect eukaryotes. Previously, two types of CRISPR/Cas effector systems were established as RNA targeting systems in vivo, FnCas9 and LshCas13a. In their experiment Zhang et al. deployed a recombinant tobacco mosaic virus containing green fluorescent protein (GFP) to infect *Nicotiana Benthamiana* leaves, combined with pCambia1300 derived plasmid vectors. To rule out the hypothesis that crCas13a inhibits infections independently was investigated by swapping Cas13a with GUS gene as well as introducing a point mutation in higher eukaryotic and prokaryotic nucleotide-binding domains at two sites. This resulted in inhibition of TMV-GFP infection, confirming that Cas13a cleavage sites are crucial for countering viral infections. Since monocot plants are also targets for viral diseases that contribute to significant crop reduction each year, there is an urgent need for viral resistance for these plants too. The first deployment of viral RNA targeting in monocot plants was reported by Zhang et al. So Zhang et al. also conducted an experiment utilizing *Southern rice black-streaked dwarf virus* (SRBSDV) to infect rice plants alongside with crRNAs targeting SRBSCV genome. The crRNAs were delivered into rice plants using agrobacterium mediation. This was followed by the process of quantification, which confirmed inhibition of infection. This discovery could mean that in the future it is possible to standardize techniques for viral resistance in many crop types [10].

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References:

1) Xiaonan Ma1 , Xiaoyan Zhang1 , Huimin Liu1 and Zhenghe Li (2020). Highly efficient DNA-free plant genome editing using virally delivered CRISPR–Cas9 State Key Laboratory of Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou, China. https://doi.org/10.1038/s41477-020-0704-5.

2) Will B. Cody and Herman B. Scholthof (2019). Plant Virus Vectors 3.0: Transitioning into Synthetic Genomics. The Annual Review of Phytopathology is online at phyto.annualreviews.org https://doi.org/10.1146/annurev-phyto-082718- 100301.

3) Patrick Schindele, Felix Wolter and Holger Puchta (2018). Transforming plant biology and breeding with CRISPR/ Cas9, Cas12 and Cas13. H. Puchta, Botanical Institute, Karlsruhe Institute of Technology. https://febs.onlinelibrary.wiley.com/doi/full/10.1002/1873-3468.13073.

4) Aili Bao, David J. Burritt, Haifeng Chen, Xinan Zhou, Dong Cao & Lam-Son Phan Tran (2019): The CRISPR/Cas9 system and its applications in crop genome editing, Critical Reviews in Biotechnology, DOI: 10.1080/07388551.2018.1554621.

5) Sergei Svitashev , Joshua K. Young , Christine Schwartz, Huirong Gao, S. Carl Falco, and A. Mark Cigan (2021). Trait Enabling Technologies, DuPont Pioneer, Johnston, Iowa 50131 ORCID IDs: 0000-0002-5926-3839 (S.S.); 0000-0002-5983-5235 (A.M.C.)

6) Gavin J Knot, Alexandra East-Seletsky, Joshua C Cofsky, James M Holton, Emeric Charles, Mitchell R O'Connell & Jennifer A Doudna (2017) . Guide-bound structures of an RNA-targeting A-cleaving CRISPR–Cas13a enzyme. Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, California, USA. doi: 10.1038/nsmb.3466.

7) Yu-Fan Chuang, Andrew J. Phipps, Fan-Li Lin, Valerie Hecht, Alex W. Hewit, Peng-Yuan Wang, Guei-Sheung Liu (2021). Approach for in vivo delivery of CRISPR/Cas system: a recent update and future prospect. Shenzhen Key Laboratory of Biomimetic Materials and Cellular Immunomodulation, China. doi: 10.1007/s00018-020-03725-2.

8) Sonal Srivastava, Dilip J. Upadhyay and Ashish Srivastava (2020). Next-Generation Molecular Diagnostics Development by CRISPR/Cas Tool: Rapid Detection and Surveillance of Viral Disease Outbreaks. 1 Amity Institute of Virology and Immunology, Amity University Uttar Pradesh, Noida, India, 2 Amity University Uttar Pradesh, Noida, India.
9) Muhammad Zuhaib Khan, Imran Amin, Amir Hameed and Shahid Mansoor (2018). CRISPR–Cas13a: Prospects for Plant Virus Resistance. National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan.
10) Tong Zhang, Yaling Zha, Jiajie Ye, Xue Cao1, Chenhui Xu1, Biao Chen1, Hong An, Yuting Jiao, Fushun Zhang, Xin Yang and Guohui Zhou (2019). Establishing CRISPR/Cas13a immune system conferring RNA virus resistance in both dicot and monocot plants. Guangdong Province Key Laboratory of Microbial Signals and Disease Control, College of Agriculture, South China Agricultural University, Guangzhou, Guangdong, China.

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CRISPR/CAS SYSTEM TO COMBAT PLANT VIRUS

Zharylkassyn G., Iksat N., Omarov R.
gauhar.zharylkassyn@gmail.com
Student of Faculty of Natural Sciences at L.N. Gumilyov Eurasian National University,
Nur-Sultan, Kazakhstan
Scientific Supervisor-Nurgul Iksat

Abstract. CRISPR-Cas and CRISPR-associated systems (CRISPR-Cas) are key immune mechanisms that allow prokaryotic species to ward off RNA and DNA viruses. CRISPR/Cas9 has broad biotechnology and basic research applications, as well as being widely used across eukaryotic species for genome engineering and functional gene analysis. Newly developed CRISPR/Cas13 systems target RNA rather than DNA and have the potential to be used for gene engineering and to combat RNA viruses. This paper provides an overview of the CRISPR/Cas system and discusses the guided RNA. We then describe different types of programs used to detect secondary structure of guided RNA in CRISPR/CAS13.

Introduction. CRISPR/Cas is an immune defense system that is currently used in biotechnology, particularly CRISPR/Cas9 for DNA targeting. CRISPR/Cas technology that targets RNA, on the other hand, is still in the works. The discovery of the type VI CRISPR / Cas system enables the discovery of RNA-guided RNA targeting. CRISPR/Cas13 is a ssRNA-targeting system with enormous potential for RNA virus targeting as well as transcript-level interference.

During the evolution of archaea and bacteria, CRISPR (clustered regularly interspaced short polyadenylic repeats)-Cas (CRISPR-associated protein) were essential for adaptive phage immunity mechanisms. The CRISPR/Cas9 and other CRISPR/Cas systems work by recognizing and binding DNA to RNA for specific nucleic acid cleavage at any desired target site at low cost. In addition to its first applications for genome editing in plants ,CRISPR-Cas is now used for genome editing in a variety of crop species, introducing traits of great value into many of them [1].

They are classified into two major classes and six types, divided further into several subtypes, as a result of their complex classification .A class 1 system (which includes types I, III, and IV) utilizes multisubunit effector complexes, whereas a class 2 system (which includes types II, V, and VI) uses a single, multidomain protein [2].

The class 2 type II CRISPR/Cas9 system of *Streptococcus pyogenes* was the first and most widely used gene-editing tool, and its mechanism is reasonably well understood [3]. Activation of this system requires an additional small RNA, a transactivating