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N.M. Kerimbek[1,2], R.T. Kenzhebekova[1,2], A.I. Kapytina[1], D.A. Gritsenko[1,2*]

[1]"Institute of Plant Biology and Biotechnology", Almaty, Kazakhstan
[2] "al-Farabi Kazakh National University", Almaty, Kazakhstan
*e-mail: d.kopytina@gmail.com

DETECTION OF GRAPEVINE FANLEAF VIRUS BY NEW GRNAS

Grapevine fanleaf virus (GFLV) is an important worldwide pathogen of grapes that leads to significant economic losses due to reduced health and productivity of the vines. In this study, a highly sensitive and specific molecular diagnostic system for GFLV was established. Synthetic control sequences were developed to detect conserved regions of the viral RNA2 genome, enabling precise detection. The performance of a CRISPR/Cas12a-based diagnostic platform integrated with Recombinase Polymerase Amplification (RPA) was validated, demonstrating sensitive and rapid detection of GFLV even at low viral loads. Although the Cas13a system was tested, its sensitivity was insufficient due to guide RNA inefficiencies and the complex structure of viral RNA, indicating the necessity for further optimization. The combination of Cas12a with RPA was demonstrated to be a powerful diagnostic approach for the detection of plant viruses, with potential for field-integrated diagnostic applications. This research provides a foundation for the development of diagnostic tools aimed at controlling GFLV and improving viticulture management approaches, thereby promoting sustainable viticulture.

Keywords: grapevine fanleaf virus, CRISPR/Cas12a, recombinase polymerase amplification, molecular diagnostics, plant virus detection.

Н.М. Керімбек[1,2], Р.Т. Кенжебекова[1,2],
А.И. Капытина[1], Д.А. Гриценко[1,2*]

[1]«Өсімдіктер биологиясы және биотехнологиясы институты», Алматы, Қазақстан
[2]«Әл-Фараби атындағы Қазақ ұлттық университеті», Алматы, Қазақстан
*e-mail: d.kopytina@gmail.com

Жаңа гРНҚ арқылы жүзімнің желпуіш-жапырақ вирусын анықтау

Жүзімнің желпуіш-жапырақ вирусы (GFLV) – жүзімнің денсаулығы мен өнімділігін төмендетуге байланысты елеулі экономикалық шығындарға әкелетін бүкіл әлемде маңызды қоздырғыш болып табылады. Бұл зерттеуде GFLV үшін жоғары сезімтал және ерекше молекулалық диагностикалық жүйе әзірленді. Вирустық RNA2 геномының сақталған аймақтарын анықтай алатын синтетикалық бақылау реттілігі жасалып, нақты анықтау мүмкіндігі қамтамасыз етілді. CRISPR/Cas12a негізіндегі диагностикалық платформаның рекомбиназалық полимеразды күшейту әдісімен (RPA) біріктірілген өнімділігі тексеріліп, вирустың аз мөлшерінде де GFLV-ді жылдам және сезімтал анықтауға мүмкіндік беретіні көрсетілді. Cas13a жүйесі де зерттелгенімен, бағыттаушы РНҚ-ның тиімсіздігі мен вирустық РНҚ-ның күрделі құрылымы себепті жеткілікті сезімталдық көрсетпегені анықталды, бұл оның әрі қарай оңтайландырылуын қажет ететінін көрсетті. Cas12a мен RPA-ны біріктіру өсімдік вирустарын анықтауға арналған қуатты диагностикалық әдіс ретінде танылып, оны далалық жағдайларда қолдану мүмкіндігі бар екені дәлелденді. Бұл зерттеу GFLV-ді бақылауға арналған диагностикалық құралдарды әзірлеу және жүзім шаруашылығын басқару тәсілдерін жақсарту үшін негіз жасайды, осылайша тұрақты жүзім шаруашылығын дамытуға ықпал етеді.

Түйін сөздер: жүзімнің желпуіш-жапырақ вирусы, CRISPR/Cas12a, рекомбиназа-полимеразды амплификация, молекулалық диагностика, өсімдік вирусын анықтау.

Н.М. Керімбек[1,2], Р.Т. Кенжебекова[1,2],
А.И. Капытина[1], Д.А. Гриценко[1,2*]
[1]«Институт биологии и биотехнологии растений», Алматы, Казахстан
[2]«Казахский национальный университет имени аль-Фараби», Алматы, Казахстан
*e-mail: d.kopytina@gmail.com

Обнаружение вируса веерной мозаики винограда с использованием новых гРНК

Вирус веерчатости листьев винограда (Grapevine fanleaf virus, GFLV) является значимым глобальным патогеном винограда, который вызывает существенные экономические потери из-за снижения здоровья и продуктивности лоз. В данном исследовании была разработана высокочувствительная и специфичная молекулярная диагностическая система для GFLV. Были созданы синтетические контрольные последовательности, которые позволяют точно выявлять консервативные участки в геноме RNA2 вируса. Производительность диагностической платформы CRISPR/Cas12a была протестирована в сочетании с методом рекомбиназной полимеразной амплификации (RPA), что продемонстрировало способность платформы к быстрому и чувствительному выявлению GFLV даже при низкой вирусной нагрузке. Хотя система Cas13a также была протестирована, её чувствительность оказалась недостаточной из-за неэффективности направляющих РНК и сложной структуры вирусной РНК, что указывает на необходимость дальнейшей оптимизации. Сочетание Cas12a и RPA было признано мощным диагностическим подходом для обнаружения растительных вирусов с потенциалом интеграции в полевых условиях. Это исследование создаёт основу для разработки диагностических инструментов, направленных на контроль GFLV и улучшение подходов к управлению виноградарством, способствуя устойчивому развитию виноградарства.

Ключевые слова: вирус веерчатости листьев винограда, CRISPR/Cas12a, рекомбиназная полимеразная амплификация, молекулярная диагностика, выявление растительных вирусов.

1. Introduction

Grapevine (Vitis spp.) is one of the most important crops in agriculture but is affected by many viruses. To date, over 90 viruses and viroids have been characterized from vineyards, many of which have a severe impact on the growth, viability, and productivity of this crop [1, 2, 3]. These viruses lower both the quality and quantity of the harvest and shorten the productive lifespan of vineyards, causing major economic damage. In grapevines, the virus is mainly transmitted in propagation via infected material or vectors such as nematodes, mealybugs, and scale insects.

Grapevine fanleaf virus (GFLV), which causes fanleaf degeneration of grapevine, is the most damaging viral diseases among these [4, 5]. This disease has been reported in over 60% of vineyard areas in France [6]. Xiphinema index [7] is the most important vector of GFLV transmission. This virus causes crop losses of up to 80% and considerably shorten the vineyard lifespan through progressive degeneration of the vines [8, 9].

Typical fanleaf degeneration symptoms include leaf discoloration (e.g., yellowing, vein banding, mosaic patterns), leaf deformation (e.g., small leaves, open petiole sinus), shoot abnormalities (e.g., short internodes, fasciation), and stunted plant growth [10]. Disease severity depends on the grapevine variety, rootstock genotype, viral strain, environmental conditions, and vineyard management practices [11].

The GFLV genome contains two positive-sense single-stranded RNAs, RNA1 and RNA2, which translate into polyproteins (P1 and P2) that are processed into functional proteins through a viral protease [12, 13]. Genetic diversity studies have indicated that GFLV has a high polymorphism rate and frequent recombination events in its genome, probably because the same plant can contain more than one viral variant [14].

Sanitary selection and certification programs targeting the most important infectious agents, such as GFLV, ampeloviruses, closteroviruses, and the phytoplasmas associated with bois noir and flavescence dorée, are recommended by the International Council for the Study of Viruses and Virus-like Diseases of Grapevine (ICVG) as a means of combatting grapevine viruses.

New developments in molecular diagnostics have resulted in novel methods harnessing isothermal amplification techniques integrated with CRISPR/Cas systems, improving the accessibility, speed, and accuracy of virus detection. These technologies allow for the early detection of viruses such as GFLV, which is important for control-

ling their spread and preventing economic losses [15, 16].

A particularly promising development is the combination of isothermal amplification techniques, including Recombinase Polymerase Amplification (RPA) or Loop-Mediated Isothermal Amplification (LAMP), and CRISPR/Cas systems. Not requiring thermal cycling, this combination enables sensitive and fast detection of viral nucleic acids and then becomes suitable for point-of-care diagnostics. The most established example of this, for instance, is the SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) platform, which achieves specific, highly sensitive, and specific RNA or DNA sequence detection with the combination of CRISPR/Cas13 and isothermal amplification [17, 18, 19].

DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter) is another representative system that uses a combination of CRISPR/Cas12a and isothermal amplification to detect plant viruses by identifying specific DNA sequences. These platforms are promising new tools to transform the field of pathogen diagnostics via rapid, accurate, and low-cost detection [17 – 20].

Though these technologies can be pathed to other organisms, their utilization for GFLV detection is in its infancy. CRISPR-based diagnostics for GFLV, when harvested or individual detection should enhance the speed of detection which in turn will allow faster management of the disease, thus preventing huge economic losses [21, 22].

2. Materials and methods

Sampling and Storage of Plant Material

In 2024, infected plant material with Grapevine fanleaf virus (GFLV) has been collected from vineyards and private farms, especially, the Turkestan region, including the village of Tulkibas. The symptoms of infection by this virus include leaf discolouration, leaf deformation and stunted growth. Samples were collected from grapevine exhibiting the symptoms of fanleaf degeneration. In order to maintain integrity, collected samples were shipped in thermal boxes, equipped with cooling packs. The samples were brought to the laboratory, where they were held at -80 °C until use.

Development of Synthetic Control Sequences

Synthetic sequences that had homology to strongly conserved regions of the GFLV genome were designed in Geneious Prime® 2024.0.5 software based on previous publications. These sequences targeted a critical region within the **RNA2 segment** of the GFLV genome, specifically the coat protein (CP) coding region, which is important for virion and transmission.

The following primers were used for amplification and detection of GFLV:

– Forward primer: CCWGACYTMTCYYTRCCAAG;

– Reverse primer: GGYTTRCACAARACDCGGAG.

These primers amplify a conserved fragment of 200–250 base pairs (bp) located between nucleotide positions 5,200–5,400 bp of the RNA2 genome segment according to the reference sequence (GCA_000860305.1). High conservation across diverse GFLV isolates in this region facilitated accurate and specific detection.

These synthetic sequences were cloned into the pMG-Amp plasmid, ordered from Macrogen (Republic of Korea), to facilitate further experimentation and the development of molecular diagnostic tools.

Cloning and Transformation

The GFLV sequences were cloned by blunt-end ligation into the pCAMBIA2300 vector. The GFLV sequences were cloned in this study under the control of the Cauliflower mosaic virus (CaMV) 35S promoter and the nopaline synthase (NOS) terminator.

Transformation of E. coli cells (Dh5α strain) was carried out using the heat shock method. Competent cells were thawed on ice for 15 minutes, followed by the addition of ligated plasmid DNA. The mixture was incubated on ice for 30 minutes. Cells were subjected to heat shock at 42°C for 1 minute, then cooled on ice for 5 minutes. A total of 250 µL of LB medium (antibiotic-free) was added to the cells, and the mixture was incubated at 37°C with shaking (160 rpm) for 1 hour. After centrifugation at 5000 rpm for 5 minutes, the supernatant was discarded, and the pellet was resuspended in 100 µL of the remaining solution.

The transformed cells were plated onto solid LB agar medium containing kanamycin (50 µg/mL) and incubated at 37°C for 16 hours. Individual colonies were transferred to 2 mL of liquid LB medium with kanamycin and incubated at 37°C with shaking (160 rpm) for 16 hours. Transformation success was confirmed using restriction digestion.

Confirmed clones were stored as glycerol stocks at -80°C. Plasmid DNA was extracted using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific), and concentrations were measured using a NanoDrop One spectrophotometer (Thermo Fisher Scientific).

RNA Extraction and Reverse Transcription

Total RNA was extracted from GFLV-infected plant material using the FastPure Plant Total RNA Isolation Kit (Vazyme) following the manufacturer's protocol. RNA quality was assessed by electrophoresis in a 1.5% agarose gel with TAE buffer and ethidium bromide staining.

For reverse transcription, the following reaction mixture (15 μL) was prepared: 0.5 mM oligo-dT primers, 0.5 mM random hexamer primers, 3 μL of total RNA. The mixture was incubated at 72°C for 10 minutes, cooled on ice for 3 minutes, then supplemented with 1X RT buffer, 0.5 mM dNTPs, and 5 U of RevertAid Reverse Transcriptase (Thermo Fisher Scientific). The reaction was carried out at 45°C for 1 hour.

Recombinase Polymerase Amplification (RPA)

RPA was performed to amplify target regions of the GFLV genome using Bsu DNA Polymerase (New England Biolabs). Primers targeting conserved regions of GFLV, as listed above, were designed using PrimedRPA software. The reaction mixture included: 1X NEBuffer™ 2, 0.2 mM primers, 50 ng of DNA template.

Reactions were incubated at 37°C for 1 hour with constant agitation. Amplification products were analyzed via electrophoresis in a 1.5% agarose gel with TAE buffer.

CRISPR/Cas12a-Based Detection

For CRISPR/Cas12a detection, the enzyme EnGen® Lba Cas12a (Cpf1) (New England Biolabs) was complexed with synthetic guide RNA (gRNA), transcribed from the pMG-Amp plasmid using the MEGAscript™ T7 Transcription Kit (Thermo Fisher Scientific). The detection mixture included: 2 μL of RPA-amplified DNA, 50 nM of the Cas12a/gRNA complex, 500 nM of single-stranded DNA fluorescent reporter. Fluorescence readings were taken on the QuantStudio 5 real-time PCR system at 37°C for 30-60 minutes.

Comparative Analysis of Detection Methods

To compare the efficiency of the CRISPR/Cas12a-based system, conventional PCR was performed. The reaction mixture included: 1X Taq buffer, 0.2 mM dNTPs, 0.2 mM primers (as listed above), 1 U of Taq polymerase. PCR products were analyzed using agarose gel electrophoresis in a 1.5% agarose gel, visualized under UV light.

3. Results and discussion

Sampling and Detection of Grapevine Fanleaf Virus (GFLV)

The study was conducted on grapevine samples collected from private farms in Turkestan region and the village Tulkibas Most plants screened displaying symptoms characteristic of Grapevine fanleaf virus (GFLV) infection, such as leaf distortion, discoloration and growth inhibition. Five of the 16 tested samples had previously been assayed and proven to be infected by GFLV.

Pathogen detection was performed using modern molecular techniques such as reverse transcription polymerase chain reaction (RT-PCR). Sensitivity and specificity of RT-PCR were both high, thereby making RT-PCR a reliable method for the diagnosis of viral infections in grapevines. Subsequent sequencing showed, with regard to the GFLV, that local strains might differ genetically, improving the knowledge of the evolution and epidemiology of the virus.

Figure 1 presents the RT-PCR results for GFLV. The successful amplification of target sequences in infected samples highlights the effectiveness of RT-PCR for detecting these viruses.

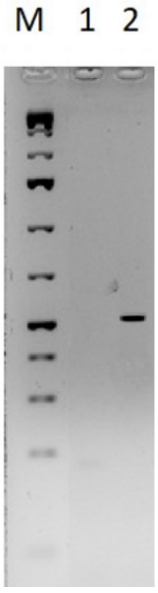

Figure 1 – The electrophoresis results of RT-PCR detection: M – DNA size marker (1 Kb Plus DNA Ladder, Invitrogen). 1– Negative control for GFLV. 2 – GFLV -infected sample.

These results confirm the specific amplification of viral sequences and the absence of nonspecific bands in negative controls, underscoring the reliability of RT-PCR for GFLV detection.

Development of Synthetic Control Sequences for GFLV Detection

To facilitate diagnostics of GFLV, synthetic control sequences were designed based on conserved

regions of the viral genome. These sequences were designed for enabling scalable and reproducible detection. The synthetic constructs were cloned into plasmids in *E. coli* cells for scalable production and molecular testing.

Figure 2 presents the design of the synthetic sequence for GFLV, with key components used for molecular cloning and diagnostic applications. The diagnostic target, identified as the Gf-con homolog conserved region, is marked with a blue bar. Two specific primers, Gf-3 and Gf-4 (green bars), are used to amplify this target region. The figure also indicates the restriction sites (PstI, SstI, SacI, AvaI, XhoI, and AluI) located further downstream that are important for cloning and sequence verification.This design allows for plasmid vector incorporation and diagnostic workflows, including CRISPR and PCR-based approaches.

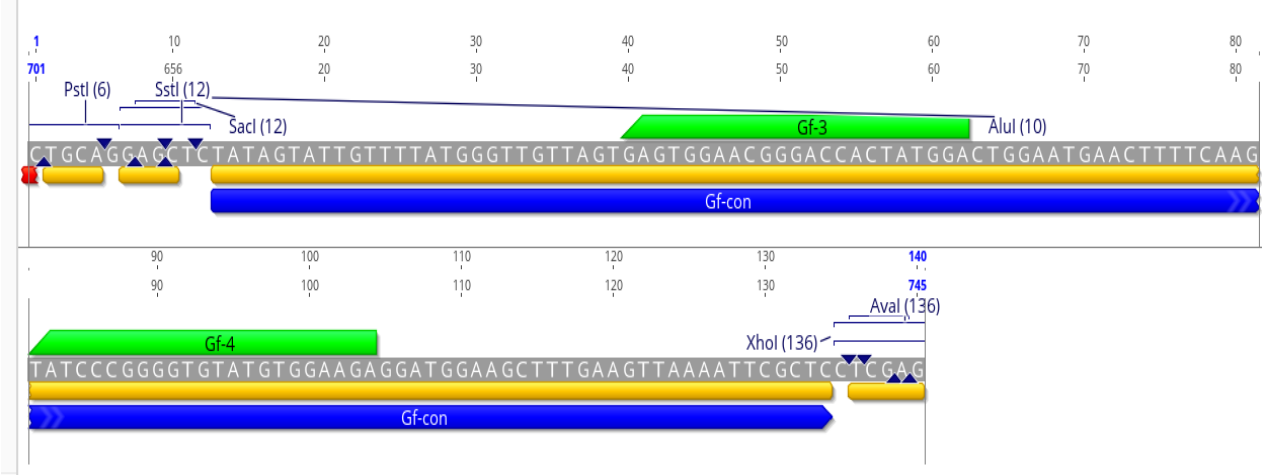

Figure 2 – A schematic representation of the synthetic sequence for GFLV

Optimization of GFLV Detection Using CRISPR/Cas Systems

Synthetic control sequences complementary to conserved regions of the GFLV genome were used to optimize detection protocols. We tested two CRISPR-based systems, Cas13a and Cas12a, to assess their sensitivity and efficacy for detecting GFLV infections.

Direct detection of GFLV RNA by the Cas13a system used RNAse Alert reporters to measure RNA cleavage activity. This approach, however, showed very low sensitivity, as pronounced fluorescence signal were obtained only at high RNA concentration. The fluorescence was observed to peak at 306.14 RFU at 10 ng/µL and 51.43 RFU at a concentration of 1 ng/µL, with concentrations of 100 pg/µL or below not showing any detectable signal. As such, these results illustrate the crude sensitivity of Cas13a type sdRNPs as presently configured. More optimizations are needed to enhance the system specificity and efficient targeting of viral RNA by for example, designing guide RNAs.

In comparison, the Cas12a system used reverse transcription and RPA (recombinase polymerase amplification) to amplify the target sequence in advance of detection. Combined with DNAse Alert reporters, this increased sensitivity dramatically. The fluorescence signals were 512.20 RFU (10 ng/µL) and 131.72 RFU (1 ng/µL), indicating the capacity to detect lower viral loads than what was achievable with Cas13a. No signals were observed with concentrations lower than 100 pg/µL, but was overall expected as the additional use of RPA was indeed able to amplify enough the target material to increase the detection limit.

The GFLV-specific RPA primers were designed based on strict criteria to minimize off-target amplifications and maximizing efficiencies for RPA reactions. Primers were 30–35 nucleotides in length, with GC content between 40% and 60%, no secondary structures, and no formation of heterodimer. The GFLV target sequence was amplified using specific forward primer (5'-CGTACGACTGATGCTGACGTGCT-3') and reverse primer (5'-GACTGACGTAGCTGACGTGACT-3').

The relative comparison of the systems confirmed the benefits of Cas12a over Cas13a for GFLV detection. Combined with RPA, Cas12a

exhibited greater reliability and sensitivity, establishing it as the diagnostic method of choice for GFLV infections. While Cas13a has a limited performance, meaning additional optimization, especially the guide RNA design, is required for GFLV, it is confirmed a better diagnostic approach in this sense.

The results of this study highlight the benefits of integrating RPA with CRISPR/Cas systems for enhanced molecular diagnostics. This detection method offers high sensitivity, specificity, and efficiency, and can be readily applied on-site, which is particularly valuable for controlling economically important grapevine viruses. An additional approach involves optimizing the Cas13a system while developing alternative protocols for a broader range of sample types to evaluate the viability of these tools across diverse contexts.

The results demonstrate the relevance of CRISPR systems to plant virus diagnostics, mirroring recent reports about their speed and sensitivity. For instance, Mahas et al. developed a CRISPR–Cas12a-based assay to detect plant DNA viruses with high sensitivity and specificity in about one hour [23]. Similarly, Aman et al. reported an efficient one-pot RT-RPA–CRISPR/Cas12a assay for plant RNA viruses, emphasizing its rapidity and sensitivity [24].

However, in this study, the Cas12a system showed the greatest sensitivity for GFLV detection when combined with RPA which was consistent with prior research. An amplification step, such as RPA, is likely implicated in the improved sensitivity associated with Cas12a-based detection methods. In contrast, the RNA-targeting Cas13a system did not incorporate an amplification step and, as a result, exhibited lower sensitivity. The limitations may be due to the guide RNA being poorly designed, or that the structure of the viral RNA is complex and has been implicated in previous studies as affecting detection efficacy. [25-27].

The development of synthetic control sequences for GFLV provided a reliable means to optimize and validate the detection assays. Such controls are essential for standardizing diagnostic tests and ensuring their accuracy and reproducibility across different laboratories and field conditions.

Conclusion

The Cas12a system along with RPA was validated as rapid, highly sensitive, and specific molecular identification strategies for the Grapevine fanleaf virus (GFLV). Its sensitivity to detect low viral loads makes it particularly appropriate for the diagnosis of GFLV infection in grapevine samples. The introduction of an additional amplification step, such as RPA, increases the detection of sensitivity and makes the Cas12a system the diagnostic of choice compared to the less sensitive Cas13a system in its present configuration.

Although the Cas13a system had limited sensitivity, we speculate that this was due to potentially subpar guide RNA design or structural complexity of the viral RNA. Future work will be centered on guide RNAs for Cas13a optimization to enhance diagnostic capability of GFLV and other plant viruses.

The Cas12a system will also undergo further confirmation on a broader sample size obtained from various geographical locations. This will enable its robustness and reliability across diverse field conditions. Furthermore, the adaptation of Cas12a-based detection techniques to other plant pathogens may enhance its applicability in agricultural diagnostics.

Synthetic control sequences were integral to this study, providing a standardized and scalable framework for molecular diagnostics. Such controls not only facilitated the fine-tunining of detection protocol but also went a long way in ensuring the reproducibility of the experiments, an essential feature in the development of diagnostic assays.

Overall, the proposed CRISPR/Cas12a platform integrated with RPA is a promising step towards robust molecular diagnostics of GFLV, contributing to faster, more sensitive, and scalable plant virus detection. In the future, we will improve the guide RNAs for both the Cas12a and Cas13a systems and test their applicability for more plant viruses. Moreover, the advancement in portable field-deployable diagnostic technologies stemming from these systems potentially transforms approaches towards plant disease management, facilitating the early detection and control of economically significant pathogens in viticulture and other high-value crops.

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References

1. Fuchs M. Grapevine viruses: Did you say more than a hundred? // Journal of Plant Pathology. – 2024. – https://doi.org/10.1007/s42161-024-01819-5.

2. Zherdev A.V., Vinogradova S.V., Byzova N.A., Porotikova E.V., Kamionskaya A.M., Dzantiev B.B. Methods for the Diagnosis of Grapevine Viral Infections: A Review // Agriculture. – 2018. – Vol. 8. – No. 12. – Article 195. https://doi.org/10.3390/agriculture8120195.

3. Basso M.F., Fajardo T.V.M., Saldarelli P. Grapevine virus diseases: economic impact and current advances in viral prospection and management // Revista Brasileira de Fruticultura. – 2017. – Vol. 39. – No. 1. – Article e-411.

4. Martelli G.P. An overview on grapevine viruses, viroids, and the diseases they cause // Grapevine Viruses: Molecular Biology, Diagnostics and Management. – Cham, Switzerland: Springer International Publishing, 2017. – P. 31–46.

5. Gambino G., Di Matteo D., Gribaudo I. Elimination of Grapevine fanleaf virus from three Vitis vinifera cultivars by somatic embryogenesis // European Journal of Plant Pathology. – 2009. – Vol. 123. – P. 57–60. https://doi.org/10.1007/s10658-008-9342-x.

6. Demangeat G., Esmenjaud D., Voisin R.R., Bidault J.M., Grenan S., Claverie M. Le court-noué de la vigne: État des connaissances sur cette maladie // Phytoma. – 2005. – No. 587. – P. 38–42.

7. Andret-Link P., Schmitt-Keichinger C., Demangeat G., Komar V., Fuchs M. The specific transmission of Grapevine fanleaf virus by its nematode vector Xiphinema index is solely determined by the viral coat protein // Virology. – 2004. – Vol. 320. – P. 12–22.

8. Legin R., Bass P., Etienne L., Fuchs M. Selection of mild virus strains of fanleaf degeneration by comparative field performance of infected grapevines // VITIS – Journal of Grapevine Research. – 2015. – Vol. 32. – No. 2. – P. 103–110. https://doi.org/10.5073/vitis.1993.32.103-110.

9. Krebelj A.J., Čepin U., Ravnikar M., et al. Spatio-temporal distribution of Grapevine fanleaf virus (GFLV) in grapevine // European Journal of Plant Pathology. – 2015. – Vol. 142. – P. 159–171. https://doi.org/10.1007/s10658-015-0600-4.

10. Vuittenez A. Variation des symptômes de la dégénérescence infectieuse de la vigne // Comptes Rendus Séances L'académie Sci. – 1956. – Vol. 243. – P. 515–517.

11. Vigne E., Komar V., Fuchs M. Comparative pathogenic effects of distinct Grapevine fanleaf virus strains on *Vitis vinifera* cvs Gewurztraminer and Chardonnay // Proceedings of the 18th Congress of the International Council for the Study of Virus and Virus-Like Diseases of the Grapevine (ICVG). – Ankara, Turkey, 7–11 September 2015. – P. 236–237.

12. Panno S., Caruso A.G., Bertacca S., Pisciotta A., Lorenzo R.D., Marchione S., Matić S., Davino S. Genetic structure and molecular variability of Grapevine fanleaf virus in Sicily // Agriculture. – 2021. – Vol. 11. – No. 496. https://doi.org/10.3390/agriculture11060496.

13. Schmitt-Keichinger C., Hemmer C., Komar V., Fuchs M. Molecular, cellular, and structural biology of Grapevine fanleaf virus // Grapevine Viruses: Molecular Biology, Diagnostics and Management. – Springer International Publishing: Cham, Switzerland, 2017. – P. 83–107.

14. Vigne E., Komar V., Fuchs M. Population structure and genetic variability within isolates of Grapevine fanleaf virus from a naturally infected vineyard in France: Evidence for mixed infection and recombination // Journal of General Virology. – 2004. – Vol. 85. – P. 2435–2445.

15. Andret-Link P., Laporte C., Valat L., Demangeat G., Vigne E., Laval V., Pfeiffer P., Stussi-Garaud C., Fuchs M. Functional analysis of the protease domain encoded by RNA2 of Grapevine fanleaf virus // Virology. – 2004. – Vol. 321. – No. 1. – P. 16–27. https://doi.org/10.1016/j.virol.2004.01.009.

16. Li Y. Naked-eye detection of Grapevine red-blotch viral infection using a plasmonic CRISPR Cas12a assay // Analytical Chemistry. – 2019. – Vol. 91. – No. 21. – P. 13875–13883. https://doi.org/10.1021/acs.analchem.9b03691.

17. Kellner M.J., Koob J.G., Gootenberg J.S., Abudayyeh O.O., Zhang F. SHERLOCK: nucleic acid detection with CRISPR nucleases // Nature Protocols. – 2019. – Vol. 14. – P. 2986–3012. https://doi.org/10.1038/s41596-019-0210-2.

18. Mustafa M.I., Makhawi A.M. SHERLOCK and DETECTR: CRISPR-Cas systems as potential rapid diagnostic tools for emerging infectious diseases // Journal of Clinical Microbiology. – 2021. – Vol. 59. https://doi.org/10.1128/jcm.00745-20.

19. Joung J., Ladha A., Saito M., Kim N.G., Woolley A.E., Segel M., Barcena C., Collins J.J., Koochaki S.H., Yan W.X., Zhang F. Detection of plant pathogens using CRISPR-based diagnostics // Nature Reviews Microbiology. – 2020. – Vol. 18. – P. 702–714. https://doi.org/10.1038/s41579-020-00422-w.

20. Gootenberg J.S., Abudayyeh O.O., Lee J.W., Essletzbichler P., Dy A.J., Joung J., Verdine V., Donghia N., Daringer N.M., Freije C.A., Myhrvold C., Bhattacharyya R.P., Livny J., Regev A., Koonin E.V., Hung D.T., Sabeti P.C., Collins J.J., Zhang F. Nucleic acid detection with CRISPR-Cas13a/C2c2 // Science. – 2017. – Vol. 356. – No. 6336. – P. 438–442. https://doi.org/10.1126/science.aam9321.

21. Chen J.S., Ma E., Harrington L.B., Da Costa M., Tian X., Palefsky J.M., Doudna J.A. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity // Science. – 2018. – Vol. 360. – No. 6387. – P. 436–439. https://doi.org/10.1126/science.aar6245.

22. Vigne E., Komar V., Fuchs M. Advances in Grapevine Fanleaf Virus diagnostics and control strategies // Journal of Plant Pathology. – 2021. – Vol. 103. – P. 9–19. https://doi.org/10.1007/s42161-020-00686-x.

23. Mahas A., Hassan N., Aman R., Marsic T., Wang Q., Ali Z., Mahfouz M.M. LAMP-coupled CRISPR–Cas12a module for rapid and sensitive detection of plant DNA viruses // Viruses. – 2021. – Vol. 13. – No. 466. https://doi.org/10.3390/v13030466.

24. Aman R., Mahas A., Marsic T., Hassan N., Mahfouz M.M. Efficient, rapid, and sensitive detection of plant RNA viruses with one-pot RT-RPA–CRISPR/Cas12a assay // Frontiers in Microbiology. – 2020. – Vol. 11. – Article 610872. https://doi.org/10.3389/fmicb.2020.610872.

25. Hak H., Ostendorp S., Reza A., Greenberg S.I., Pines G., Kehr J., Spiegelman Z. Rapid on-site detection of crop RNA viruses using CRISPR/Cas13a // Journal of Experimental Botany. – 2024. – Article erae495. https://doi.org/10.1093/jxb/erae495.

26. Li Y., Wang X., Xu R., Zhang T., He L., Liu W. Establishment of RT-RPA-Cas12a assay for rapid and sensitive detection of human rhinovirus B // BMC Microbiology. – 2023. – Vol. 23. – Article 333. https://doi.org/10.1186/s12866-023-03096-1.

27. Krohannon A., Srivastava M., Rauch S., Schmitt L., Cohen A., Daley A., Gootenberg J.S. CASowary: CRISPR-Cas13 guide RNA predictor for transcript depletion // BMC Genomics. – 2022. – Vol. 23. – Article 172. https://doi.org/10.1186/s12864-022-08366-2.

References

1. Aman R., Mahas A., Marsic T., Hassan N., Mahfouz M.M. (2020) Efficient, rapid, and sensitive detection of plant RNA viruses with one-pot RT-RPA–CRISPR/Cas12a assay. *Frontiers in Microbiology*, vol. 11, article 610872. https://doi.org/10.3389/fmicb.2020.610872.

2. Andret-Link P., Laporte C., Valat L., Demangeat G., Vigne E., Laval V., Pfeiffer P., Stussi-Garaud C., Fuchs M. (2004) Functional analysis of the protease domain encoded by RNA2 of Grapevine fanleaf virus. *Virology*, vol. 321, no. 1, pp. 16–27. https://doi.org/10.1016/j.virol.2004.01.009.

3. Andret-Link P., Schmitt-Keichinger C., Demangeat G., Komar V., Fuchs M. (2004) The specific transmission of Grapevine fanleaf virus by its nematode vector Xiphinema index is solely determined by the viral coat protein. *Virology*, vol. 320, pp. 12–22.

4. Basso M.F., Fajardo T.V.M., Saldarelli P. (2017) Grapevine virus diseases: economic impact and current advances in viral prospection and management. *Revista Brasileira de Fruticultura*, vol. 39, no. 1, article e-411.

5. Chen J.S., Ma E., Harrington L.B., Da Costa M., Tian X., Palefsky J.M., Doudna J.A. (2018) CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, vol. 360, no. 6387, pp. 436–439. https://doi.org/10.1126/science.aar6245.

6. Demangeat G., Esmenjaud D., Voisin R.R., Bidault J.M., Grenan S., Claverie M. (2005) Le court-noué de la vigne: État des connaissances sur cette maladie. *Phytoma*, no. 587, pp. 38–42.

7. Fuchs M. (2024) Grapevine viruses: Did you say more than a hundred? *Journal of Plant Pathology*, https://doi.org/10.1007/s42161-024-01819-5.

8. Gambino G., Di Matteo D., Gribaudo I. (2009) Elimination of Grapevine fanleaf virus from three Vitis vinifera cultivars by somatic embryogenesis. *European Journal of Plant Pathology*, vol. 123, pp. 57–60. https://doi.org/10.1007/s10658-008-9342-x.

9. Gootenberg J.S., Abudayyeh O.O., Lee J.W., Essletzbichler P., Dy A.J., Joung J., Verdine V., Donghia N., Daringer N.M., Freije C.A., Myhrvold C., Bhattacharyya R.P., Livny J., Regev A., Koonin E.V., Hung D.T., Sabeti P.C., Collins J.J., Zhang F. (2017) Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, vol. 356, no. 6336, pp. 438–442. https://doi.org/10.1126/science.aam9321.

10. Hak H., Ostendorp S., Reza A., Greenberg S.I., Pines G., Kehr J., Spiegelman Z. (2024) Rapid on-site detection of crop RNA viruses using CRISPR/Cas13a. *Journal of Experimental Botany*, p. 495. https://doi.org/10.1093/jxb/erae495.

11. Joung J., Ladha A., Saito M., Kim N.G., Woolley A.E., Segel M., Barcena C., Collins J.J., Koochaki S.H., Yan W.X., Zhang F. (2020) Detection of plant pathogens using CRISPR-based diagnostics. *Nature Reviews Microbiology*, vol. 18, pp. 702–714. https://doi.org/10.1038/s41579-020-00422-w.

12. Kellner M.J., Koob J.G., Gootenberg J.S., Abudayyeh O.O., Zhang F. (2019) SHERLOCK: nucleic acid detection with CRISPR nucleases. *Nature Protocols*, vol. 14, pp. 2986–3012. https://doi.org/10.1038/s41596-019-0210-2.

13. Krehannon A., Srivastava M., Rauch S., Schmitt L., Cohen A., Daley A., Gootenberg J.S. (2022) CASowary: CRISPR-Cas13 guide RNA predictor for transcript depletion. *BMC Genomics*, vol. 23, article 172. https://doi.org/10.1186/s12864-022-08366-2.

14. Krebelj A.J., Čepin U., Ravnikar M., et al. (2015) Spatio-temporal distribution of Grapevine fanleaf virus (GFLV) in grapevine. *European Journal of Plant Pathology*, vol. 142, pp. 159–171. https://doi.org/10.1007/s10658-015-0600-4.

15. Legin R., Bass P., Etienne L., Fuchs M. (2015) Selection of mild virus strains of fanleaf degeneration by comparative field performance of infected grapevines. *VITIS – Journal of Grapevine Research*, vol. 32, no. 2, pp. 103–110. https://doi.org/10.5073/vitis.1993.32.103-110.

16. Li Y., Wang X., Xu R., Zhang T., He L., Liu W. (2023) Establishment of RT-RPA-Cas12a assay for rapid and sensitive detection of human rhinovirus B. *BMC Microbiology*, vol. 23, article 333. https://doi.org/10.1186/s12866-023-03096-1.

17. Li Y. (2019) Naked-eye detection of Grapevine red-blotch viral infection using a plasmonic CRISPR Cas12a assay. *Analytical Chemistry*, vol. 91, no. 21, pp. 13875–13883. https://doi.org/10.1021/acs.analchem.9b03691.

18. Mahas A., Hassan N., Aman R., Marsic T., Wang Q., Ali Z., Mahfouz M.M. (2021) LAMP-coupled CRISPR–Cas12a module for rapid and sensitive detection of plant DNA viruses. *Viruses*, vol. 13, no. 466. https://doi.org/10.3390/v13030466.

19. Martelli G.P. (2017) An overview on grapevine viruses, viroids, and the diseases they cause. Grapevine Viruses: Molecular Biology, Diagnostics and Management. *Cham, Switzerland: Springer International Publishing*, pp. 31–46.

20. Mustafa M.I., Makhawi A.M. (2021) SHERLOCK and DETECTR: CRISPR-Cas systems as potential rapid diagnostic tools for emerging infectious diseases. *Journal of Clinical Microbiology*, vol. 59. https://doi.org/10.1128/jcm.00745-20.

21. Panno S., Caruso A.G., Bertacca S., Pisciotta A., Lorenzo R.D., Marchione S., Matić S., Davino S. (2021) Genetic structure and molecular variability of Grapevine fanleaf virus in Sicily. *Agriculture*, vol. 11, article 496. https://doi.org/10.3390/agriculture11060496.

22. Schmitt-Keichinger C., Hemmer C., Komar V., Fuchs M. (2017) Molecular, cellular, and structural biology of Grapevine fanleaf virus. Grapevine Viruses: Molecular Biology, Diagnostics and Management. *Cham, Switzerland: Springer International Publishing*, pp. 83–107.

23. Vigne E., Komar V., Fuchs M. (2004) Population structure and genetic variability within isolates of Grapevine fanleaf virus from a naturally infected vineyard in France: Evidence for mixed infection and recombination. *Journal of General Virology*, vol. 85, pp. 2435–2445.

24. Vigne E., Komar V., Fuchs M. (2015) Comparative pathogenic effects of distinct Grapevine fanleaf virus strains on Vitis vinifera cvs Gewurztraminer and Chardonnay. *Proceedings of the 18th Congress of the International Council for the Study of Virus and Virus-Like Diseases of the Grapevine (ICVG)*, Ankara, Turkey, 7–11 September, pp. 236–237.

25. Vigne E., Komar V., Fuchs M. (2021) Advances in Grapevine Fanleaf Virus diagnostics and control strategies. *Journal of Plant Pathology*, vol. 103, pp. 9–19. https://doi.org/10.1007/s42161-020-00686-x.

26. Vuittenez A. (1956) Variation des symptômes de la dégénérescence infectieuse de la vigne. *Comptes Rendus Séances L'académie Sci.*, vol. 243, pp. 515–517.

27. Zherdev A.V., Vinogradova S.V., Byzova N.A., Porotikova E.V., Kamionskaya A.M., Dzantiev B.B. (2018) Methods for the Diagnosis of Grapevine Viral Infections: A Review. *Agriculture*, vol. 8, no. 12, article 195. https://doi.org/10.3390/agriculture8120195.

Information about authors:

Kerimbek Nazym – PhD student of the Faculty of Biology and Biotechnology at Al-Farabi Kazakh National University, junior researcher at the Laboratory of Molecular Biology, RSE on REM «Institute of Plant Biology and Biotechnology» CS MSHE RK, (Almaty, Kazakhstan, e-mail: nazymkerimbek@gmail.com)

Kenzhebekova Roza – PhD student of the Faculty of Biology and Biotechnology at Al-Farabi Kazakh National University, researcher at the Laboratory of Molecular Biology, RSE on REM "Institute of Plant Biology and Biotechnology" CS MSHE RK (Almaty, Kazakhstan, e-mail: rozakenzhebekova344@gmail.com).

Kapytina Anastasiya – junior researcher at the Laboratory of Molecular Biology, RSE on REM "Institute of Plant Biology and Biotechnology" CS MSHE RK (Almaty, Kazakhstan, e-mail: anastasiya.kapytina@mail.ru).

Gritsenko Dilyara (corresponding author) – PhD, Associate Professor, Head of the Laboratory of Molecular Biology, RSE on REM "Institute of Plant Biology and Biotechnology" CS MSHE RK (Almaty, Kazakhstan, e-mail: d.kopytina@gmail.com).

Авторлар туралы мәлімет:

Керімбек Назым – Әл-Фараби атындағы Қазақ ұлттық университетінің биология және биотехнология факультетінің PhD докторанты, ҚР ҒЖБМ ҒК "Өсімдіктер биологиясы және биотехнологиясы институты" ШЖҚ РМК молекулалық биология зертханасының кіші ғылыми қызметкері (Алматы, Қазақстан, e-mail: nazymkerimbek@gmail.com).

Кенжебекова Роза – Әл-Фараби атындағы Қазақ ұлттық университетінің биология және биотехнология факультетінің PhD докторанты, ҚР ҒЖБМ ҒК "Өсімдіктер биологиясы және биотехнологиясы институты" ШЖҚ РМК молекулалық биология зертханасының ғылыми қызметкері (Алматы, Қазақстан, e-mail: rozakenzhebekova344@gmail.com).

Капытина Анастасия – ҚР ҒЖБМ ҒК "Өсімдіктер биологиясы және биотехнологиясы институты" ШЖҚ РМК молекулалық биология зертханасының кіші ғылыми қызметкері (Алматы, Қазақстан, e-mail: anastasiya.kapytina@mail.ru).

Гриценко Диляра (сәйкес автор) – PhD, доцент, ҚР ҒЖБМ ҒК "Өсімдіктер биологиясы және биотехнологиясы институты" ШЖҚ РМК молекулалық биология зертханасының меңгерушісі (Алматы, Қазақстан, e-mail: d.kopytina@gmail.com).

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