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Induced RNA interference and its impact on potato virus amplification in plants

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Abstract. Viral diseases in potato crops, especially Potato virus Y (PVY), Potato leafroll virus (PLRV), Potato virus M (PVM), and Potato spindle tuber viroid (PSTVd), are particularly difficult to control and represent an important problem for worldwide agriculture. In this study, we assessed the antiviral potency of RNA interference (RNAi) and CRISPR/Cas13 technologies in lowering viral titers and preventing pathogenesis.

Potato plants were inoculated with individual and combined viral pathogens and subsequently treated with constructs containing gRNA, sense, and antisense sequences used for both RNAi and Cas13-mediated degradation of the viral RNA. Quantitative PCR (qPCR) was utilized to measure RNA levels, and disease progression was observed for three weeks. Expression of Cas13 was confirmed by fluorescence microscopy and Western blot. Results of the RNAi constructs lowered levels of viral RNA up to 85% for PVY and 78% for PLRV. CRISPR/Cas13 constructs yielded even greater suppression rates (≥90% in some treatments) with marked symptom alleviation. Synergistic effects of constructs targeting multiple targets were observed with the greatest decreases in viral loads and disease severity. The differences were statistically significant ($p < 0.05$) between treated and control plants. This research demonstrates that RNAi and CRISPR/Cas13 can enhance potato resistance to viral infections. Providing a scalable, transgene-free approach to disease control, these methods contribute to sustainable agriculture and global food security.

Keywords: potato virus, RNA interference, CRISPR/Cas13, gene editing, virus suppression, molecular tools, sustainable agriculture, transgene-free technology

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Introduction

Potatoes *(Solanum tuberosum)* are a key staple crop worldwide and provide macronutrients and micronutrients for billions of people. The production of potatoes is threatened by a wide range of viral pathogens, such as Potato leafroll virus (PLRV), Potato virus Y (PVY), Potato virus M (PVM), and the viroid - Potato spindle tuber viroid (PSTVd). These infectious agents not only reduce tuber yield but negatively affect processed harvest tubers, making it critically important to protect potato plants from the negative impact of these relevant phytopathogens. This results in significant financial losses for farmers and agribusinesses across the globe. [1-10].

The management of viral diseases in potato crops remains a significant challenge due to the absence of effective chemical treatments and the rapid evolution of viral strains. Traditional approaches, such as breeding for resistance and vector control, are often labor-intensive and insufficient to counter the fast-paced emergence of new viral variants. Consequently, there is an urgent need for innovative strategies to protect potato crops from viral infections [11-14].

RNA interference (RNAi) and CRISPR/Cas systems have emerged as powerful tools for plant disease management. RNAi exploits the plant's natural defense mechanisms to silence specific viral genes, whereas CRISPR/Cas systems enable precise and programmable targeting of viral genomes for degradation. Among these, the Cas13 family of RNA-targeting enzymes has shown remarkable promise in combating RNA viruses due to its specificity and efficiency [15-17].

We investigated the effectiveness of RNA interference (RNAi) and CRISPR/Cas13 technologies on inhibiting the replication of key potato viral pathogens. Multiple studies have shown that both Cas13 as well as RNA constructs that target the gRNA (RNAi) are effective in reducing the severity of viral infections in potato plants [18-21]. We examined their capacity to reduce viral loads and symptoms in potato plants via probe- and gRNA-directed Cas13 constructs and sense/antisense RNAi constructs. This study on the use of such technologies for the management of viral infections has the potential to develop new and transgene-free, sustainable solutions for the protection of potato crops.

Here, we provide an overview of the design, implementation, and utility of these advanced molecular tools. We evaluate their potential benefits, limitations, and implications for enhancing global food security as agricultural challenges intensify.

Materials and research methods

Plant Materials and Experimental Design

The study utilized 51 economically significant potato cultivars (Solanum tuberosum), selected for their high agronomic traits and resistance potential. Virus-free plants were produced from the apical meristems of sprouted potato tubers (3-5 cm). All sprouts were thoroughly surface-sterilized before excision to ensure they were free from contamination. Details of the initial cleaning procedures, including detergent solutions containing Tween-20, and treatment with sterilizing agents such as ethanol, mercuric chloride, and hydrogen peroxide (Table 1), are provided. After sterilization, explants were washed 3-5 times with autoclaved distilled water to remove residual sterilizing agents.

Table 1

Variants of explant sterilization

No. Sterilization Option	Sterilizing Agent	Sterilization Time
1	70% Ethanol	20 s
		30 s
		50 s
2	0.1% Mercuric Chloride	1 minute
		2 minutes
		3 minutes
3	10% Hydrogen Peroxide	2 minutes
		5 minutes
		8 minutes

Sterilized plant explants were placed on Murashige and Skoog (MS) medium supplemented with sucrose, agar, and necessary vitamins. The pH prior to autoclaving was buffered to 5.7. Seven variations of growth media with different concentrations of plant growth regulators, including auxins, gibberellins, and cytokinins, were tested to optimize shoot regeneration (Table 2). Cultivation conditions were set to 25 ± 2°C under a 16-hour photoperiod with 8 hours of darkness using a climate chamber (Binder KBWF 240, Tuttlingen, Germany).

Table 2

Variants of growth media supplemented with phytohormones

Variants of MS Media	Indoleacetic Acid (IAA)	Indole-3-Butyric Acid (IBA)	Gibberellic Acid (GA)	Kinetin	6-Benzylaminopurine (6-BAP)
MC1	0.1	-	0.3	-	-
MC2	-	0.1	0.3	-	-
MC3	0.05	0.05	-	-	-
MC4	-	-	0.3	-	1
MC5	-	-	0.3	1	-
MC6	0.1	-	-	-	1
MC7	-	0.1	-	-	1

RNA Extraction and Pathogen Detection

Total RNA was extracted from 250 mg of plant tissue following a modified CTAB protocol [22]. Lysis was performed by homogenizing liquid nitrogen-ground plant tissues with CTAB buffer containing polyvinylpyrrolidone (PVP) and β-mercaptoethanol. After a 30-minute incubation at 65°C with constant shaking, chloroform extraction was performed. RNA was precipitated overnight with lithium chloride at 4°C, pelleted by centrifugation, washed with 70% ethanol,

air-dried, and dissolved in nuclease-free water. RNA integrity was assessed on 1.5% agarose gels, and concentrations were measured using a NanoDrop One spectrophotometer (Thermo Fisher Scientific).

All reverse transcription reactions were performed in 15 μL volumes containing oligo(dT) and random hexamer primers, dNTPs, and RevertAid Reverse Transcriptase (Thermo Fisher Scientific). Pathogen-specific primers for PVY, PLRV, PVM, and PSTVd detection were used for PCR (Table 3).

Table 3

Specific primers for pathogen detection

Primers for Detection	Forward Primer Sequence (5'→3')	Reverse Primer Sequence (5'→3')	Source
PVM (202 bp)	CGTACAACAGGCCGTCCAT	CGCGGATCCAGGAATACGG	[22]
PLRV (249 bp)	GCCGCTCAAGAAGACTGGAG	GGGGGTCCAACCTATAACGGAT	[22]
PVY (535 bp)	GCATCCAGTCAAACCGGAAC	GCATACGCGCTCTAACCCAC	[22]
PSTVd (123 bp)	ACCCTTCCTTTCTTCTGGGTG	GAAAAGGCGGTTCTCGGGAG	[23]

Virus and Viroid Inoculation

Potato plants were inoculated with the following pathogens: Potato leafroll virus (PLRV), Potato virus Y (PVY), Potato virus M (PVM), and Potato spindle tuber viroid (PSTVd). Infections were carried out both individually and in combination, including six different combinations (PVY + PVM, PVM + PLRV, PVY + PVM + PSTVd, PLRV+PVY, PSTVd+PVY, PLRV+PVY+PSTVd), to clearly determine any synergistic or antagonistic effects.

For mechanical inoculation, infected tissue (0.01 g) was homogenized in 5 mL of 0.06 M phosphate buffer (pH 7.0). To facilitate pathogen entry, an abrasive material was applied, and the leaves were lightly rubbed before applying the homogenate. Plants were grown under a 16-hour light/8-hour dark photoperiod at 18–25°C. Infection symptoms were monitored twice a week for at least three weeks post-inoculation [23].

CRISPR/Cas Constructs

Bioinformatic tools were used to identify conserved genomic regions of the viruses and viroids suitable for targeting by the guide RNA (gRNA) sequences. Furthermore, the first RNA sequences developed to induce RNA interference were sense and antisense. The sequences were synthesized and cloned into pBluescript II KS(+) intermediate vectors driven by particular promoters (AtU6 for gRNA and CaMV 35S for sense and antisense sequences). Cas13d self-fused to GFP subcloned into the binary vector pCambia2300.

Restriction enzymes XmaI and XbaI were used to digest the intermediate constructs to facilitate ligation into binary vectors. The recombinant constructs were initially transformed into E. coli DH5α for plasmid amplification and later electroporated into Agrobacterium tumefaciens EHA105 using Gene Pulser Xcell (Bio-Rad) (Table 4).

Table 4

Constructs used for agroinfiltration of infected plants

Variant number	Genetic constructs for agroinfiltration carrying guide RNA, sense, and antisense sequences	Viruses individually and in combinations for plant inoculation
1	PVY_T1_T2	PVY
2	PVY_S_AS	
3	PLRV_T1_T2	PLRV
4	PLRV_S_AS	
5	PVM_T1_T2	PVM
6	PVM_S_AS	
7	PSTVd _T1_T2	PSTVd
8	PVM_T1_T2 +PLRV_T1_T2	PVM + PLRV
9	PLRV_S_AS + PVM _S_AS	
10	PLRV_T1_T2 + PVY_T1_T2	PLRV + PVY
11	PLRV_S_AS+ PVY_S_AS	
12	PVY_T1_T2 +PVM_T1_T2	PVY+ PVM
13	PVY_S_AS +PVM_ S_AS	
14	PSTVd_T1_T2 + PVY_T1_T2	PSTVd + PVY
15	PVM_T1_T2 +PVY_T1_T2+PSTVd_T1_T2	PVM + PVY + PSTVd
16	PLRV_T1_T2 + PVY_T1_T2+PSTVd _T1_T2	PLRV + PVY + PSTVd

Agroinfiltration

Agrobacterium suspensions were made in infiltration buffer (10 mM MgCl2, 10 mM MES, 200 μM acetosyringone), and the OD600 was diluted to 0.5. Agroinfiltration was performed by injecting the suspensions into the intercellular spaces of leaves using a needleless syringe. Young plants with 5–6 leaves were selected, and four opposite-positioned leaves were infiltrated per plant. Constructs targeting individual pathogens and combinations were tested. Plants were grown under the same conditions as inoculated plants, and leaf samples were collected 3–5 days post-infiltration.

Protein Expression Analysis

Fluorescence microscopy and Western blotting confirmed the expression of Cas13d-GFP fusion proteins. Fluorescence imaging was performed using an EVOS™ M5000 Imaging System with corresponding filters and objectives (10x, 20x, 40x). The proteins were extracted with standard lysis buffer, separated using 7.5% SDS-PAGE gels, and transferred to PVDF membranes by semi-dry blotting. Primary monoclonal anti-GFP antibodies (Santa Cruz Biotechnology) and secondary goat anti-mouse antibodies were used to detect GFP-tagged proteins. Blots were developed with NBT/BCIP substrates.

qPCR Analysis

The expression levels of gRNA, sense, and antisense RNA, and viral RNA loads were quantified using qPCR. Reactions were performed in 20 µL volumes with Luna Universal qPCR Master Mix (NEB) and primers specific to each target (Tables 5 and 6). Thermal cycling was conducted on a QuantStudio 5 system with an initial denaturation step (95°C, 3 min), followed by 40 cycles of denaturation (95°C, 15 s), annealing (60°C, 20 s), and extension (72°C, 40 s). Three biological replicates with technical duplicates were analyzed for each construct and pathogen combination. Relative expression levels and viral loads were calculated using the ΔΔCt method, normalized to GAPDH as an internal control.

Statistical Analysis

Data were analyzed using the R statistical programming environment. Paired t-tests were employed to compare viral loads and construct expression levels between treated and control plants. Significance was set at $p < 0.05$. Statistical summaries were generated using the ggplot2 and dplyr packages in R.

Table 5

Sequences of specific primers for confirming gRNA expression, sense, and antisense sequences

Primer Name	Primer Sequence (5'→3')
PVM-gRNA-1-f	AACCCCTACCAACTGGTC
PVM-gRNA-probe	TTGAAACACCAAATACACATACCATCCA
PVM-gRNA-1-r	TGGAGTGATTGATAGATTGTTG
PLRV-gRNA-1-f	AACCCCTACCAACTGGTC
PLRV-gRNA-probe	AACTATTCCATCCTTGAATGCCGGA
PLRV-gRNA-1-r	GTCCGGCATTCAAGGATG
PVY-gRNA-1-f	AACCCCTACCAACTGGTC
PVY-gRNA-probe	AACTATTCCATCCTTGAATGCCGGA
PVY-gRNA-1-r	GTCCGGCATTCAAGGATG
PVY-gRNA-1-f	AACCCCTACCAACTGGTC
PVY-gRNA-probe	GGGTTTGAAACGTGAAACCCCTGTTTC
PVY-gRNA-1-r	TCCCGCGGAAACAGGG
PVM_as_f	GTAGCTTAATTCGCAGATTG
PVM_as_probe	GACAGTGCGTCTCTGGCATA
PVM_as_r	CTTGATCCCAATTCCCACTCA
PVY_as_f	ATTTGCTTGAGTATGTCCA
PVY_as_probe	CGGATGGCAATCGACATAGG
PVY_as_r	ATTCATCACAGTTGGCATCT
PLRV_as_f	CCAGAGGAAACAAAGTGATCA
PLRV_as_probe	TGCAAGGCTCCATAGAGACA
PLRV_as_r	GGAGGCATTGTCAGAAATG

Results Validation

Construct efficacy was assessed by analyzing viral RNA suppression, symptom alleviation, and construct expression levels. Constructs with sense and antisense sequences exhibited superior RNA interference activation, while gRNA-Cas13 combinations effectively targeted viral RNA, demonstrating the potential of this dual strategy for pathogen control.

Table 6

Specific primer sequences for viral pathogens and viroid

Pathogen Name	Detection Primers	Primer Sequence (5'→3')	Source
PLRV	PLRV-F PLRV-R PLRV probe	5'AAAGCCGAAAGGTGATTAGGC3' 5'CCTGGCTACACAGTCGCGT3' 5'/Cy5/CTCAACGCCTGCTAGAGACCGTCGAAA/BHQ-2/3'	[23]
PVY	PVY – F PVY – R PVY probe	5'GGGTTTAGCGCGTTATGCC3' 5'TCTTGTGTACTGATGCCACCG3' 5'/HEX/ CAGTGAGGGCTAGGGAAGCGCACA/BHQ-1/3'	
PVM	PVM – F PVM – R PVM probe	5'CCATAGAAGCTCTCAGCCGG3' 5' TTCATGCCACCAGTGACCTC3' 5'/6-FAM/ ATACTGCTGCAGTCCAACCC/BHQ-1/3'	
PSTVd	PSTVd – F PSTVd[26] – R PSTVd probe	5'GCCGAAACAGGGTTTTCACC3' 5'GTTTCCACCGGGTAGTAGCC3' 5'/6-FAM/ TTCTTCGGGTGTCCTTCCTC/BHQ-1/3'	
GAPDH (internal control)	GAPDH – F GAPDH – R GAPDH probe	5'TAGCTGCACCACTAACTGCC3' 5'TGCCTTCGGATTCCTCCCTA3' 5'/6-FAM/ TTCCGTGTCCCAACCGTTGA/BHQ-1/3'	

Results

Suppression of viral load using RNAi and CRISPR/Cas13 constructs

Significantly lower levels of viral RNA were detected in potato plants treated with RNAi and CRISPR/Cas13-based constructs (Table 7). Constructs targeting PVY (PVY_T1_T2) achieved an 85% reduction in viral RNA as measured by qPCR (ΔCt = -3.2). Similarly, PLRV_T1_T2 and PVM_T1_T2 constructs resulted in 78% and 82% reductions in viral RNA levels, respectively. Dual-target constructs such as PLRV_S_AS + PVY_S_AS exhibited synergistic effects, with viral load reductions exceeding 90% ($p < 0.001$). Combining RNAi and CRISPR/Cas13 increases efficiency of viral suppression (4), according to data (Figure 1).

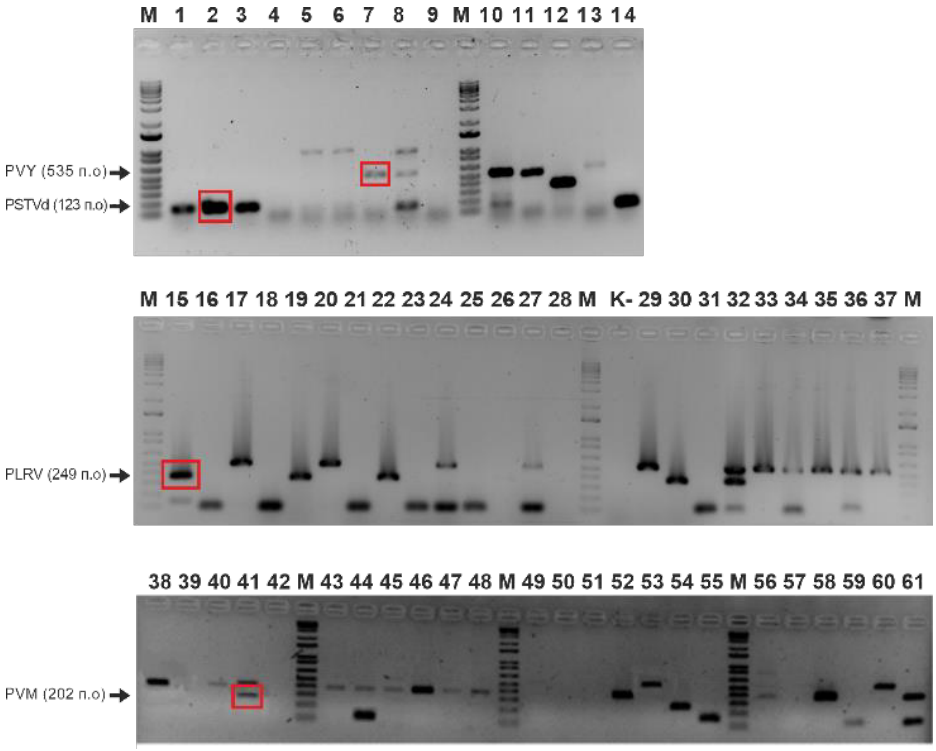
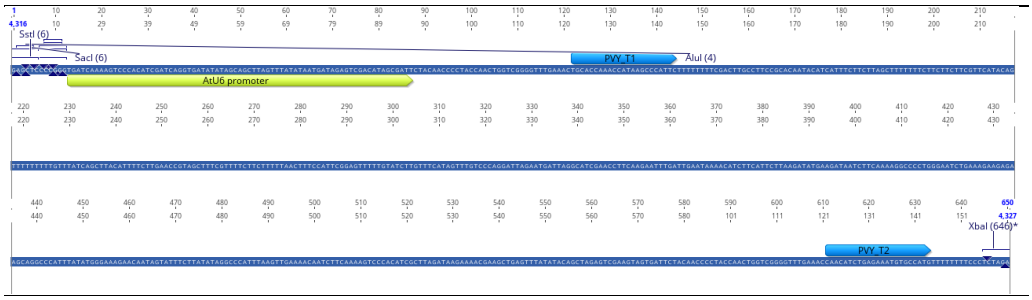
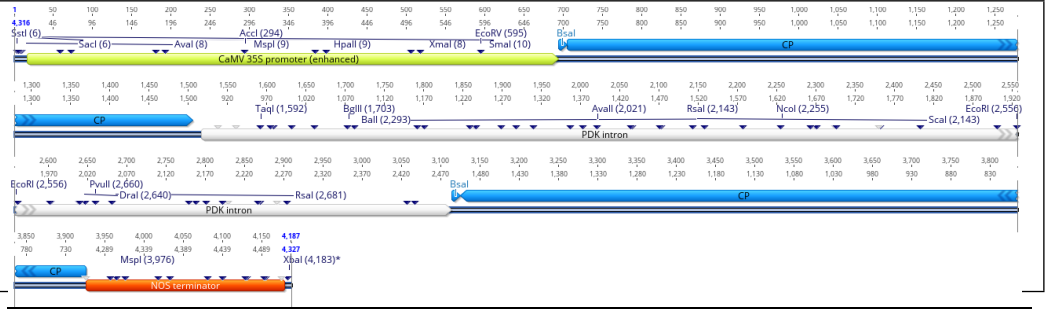
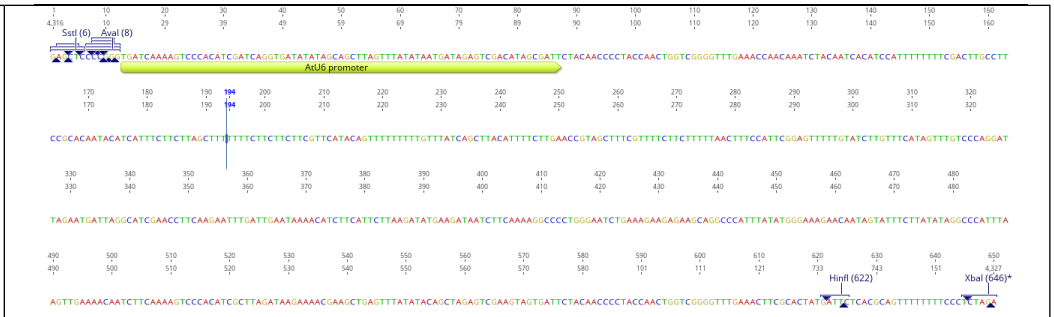
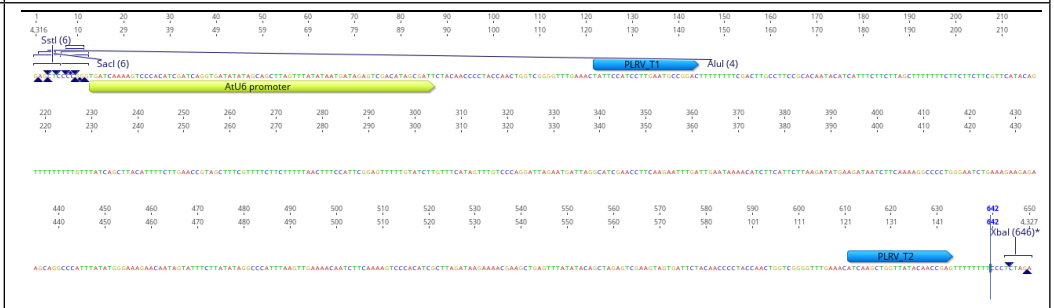
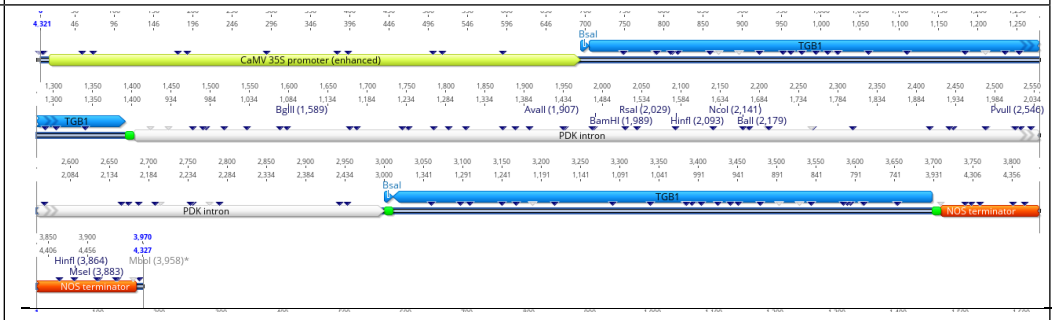
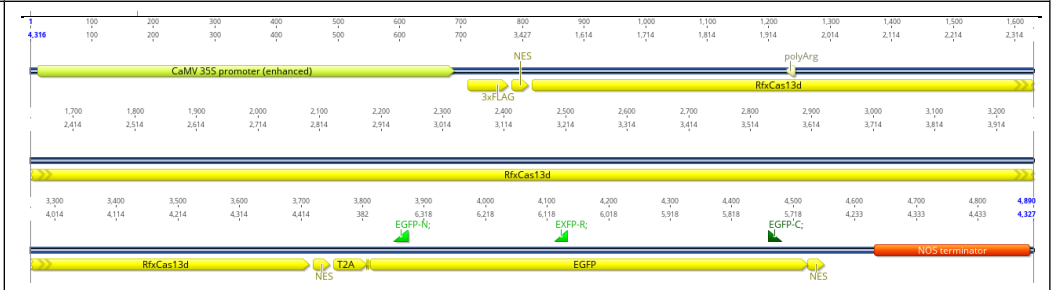


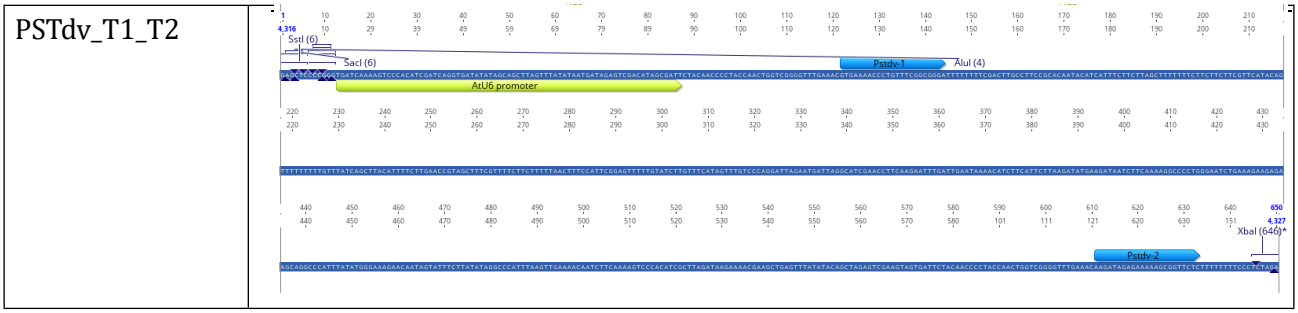
Figure 1. Detection of PVY, PLRV, PVM viruses, and PSTVd viroid in potato samples after inoculation

Table 7

Constructs Carrying Guide RNA, Sense and Antisense Sequences, and the Cas13 Gene

Name of Genetic Constructs	Schematic Representation
PVY_T1_T2	
PVY_S_AS	

PVM_T1_T2	
PLRV_T1_T2	
PLRV_S_AS	
PVM_ S_AS	
Cas13d_GFP	



Validation of Cas13d-GFP Expression and Localization

The expression and functionality of the Cas13d-GFP fusion protein were validated through fluorescence microscopy and Western blotting (Figures 2, 3). Fluorescence microscopy confirmed the proper cytoplasmic localization of the fusion protein, while Western blot analysis showed clear protein bands corresponding to Cas13d-GFP at the expected molecular weight. These results confirm efficient expression and retention of functionality in treated potato plants.

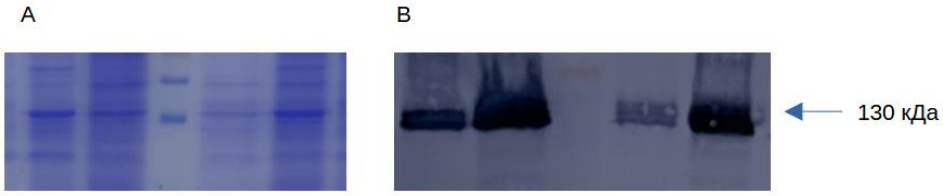


Figure 2. Analysis of Cas13d Protein Expression: (A) Results of Cas13d Protein Electrophoresis in Polyacrylamide Gel; (B) Immunoblotting of Cas13d Protein Using Antibodies to the 3xFLAG Tag

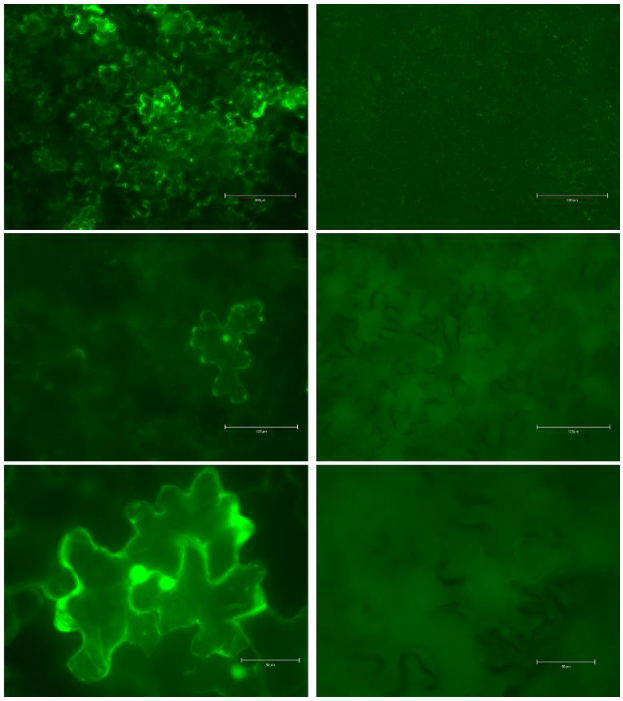


Figure 3. Results of Fluorescence Microscopy: Analysis of eGFP Protein Expression

Alleviation of Disease Symptoms

Plants that received the CRISPR/Cas13 constructs showed a significant reduction of disease symptoms. For instance, PVY-infected plants exhibit less necrosis and leaf deformation, while PLRV-infected plants exhibit minimized leaf curling and chlorosis. Multi-target constructs (e.g., PLRV + PVY + PSTVd) demonstrated the most significant impact, almost fully eliminating symptoms and restoring plant health. These results highlight the therapeutic potential of CRISPR/Cas13 and RNAi technologies for use against viral diseases.

Comparative efficacy of RNAi and CRISPR/Cas13 systems

The study revealed differences in the onset of viral suppression between RNAi and CRISPR/Cas13 constructs. CRISPR/Cas13 constructs as well as RNAi constructs were able to detectably suppress viral RNA, but were faster, with suppression detectable within seven days post-treatment for CRISPR/Cas13 constructs compared with ten days post-treatment for RNAi constructs (Figure 4). Furthermore, CRISPR/Cas13 exhibited higher specificity in targeting conserved viral regions, reducing the risk of pathogen escape through mutations. These findings position CRISPR/Cas13 as a more precise and efficient tool for pathogen control.

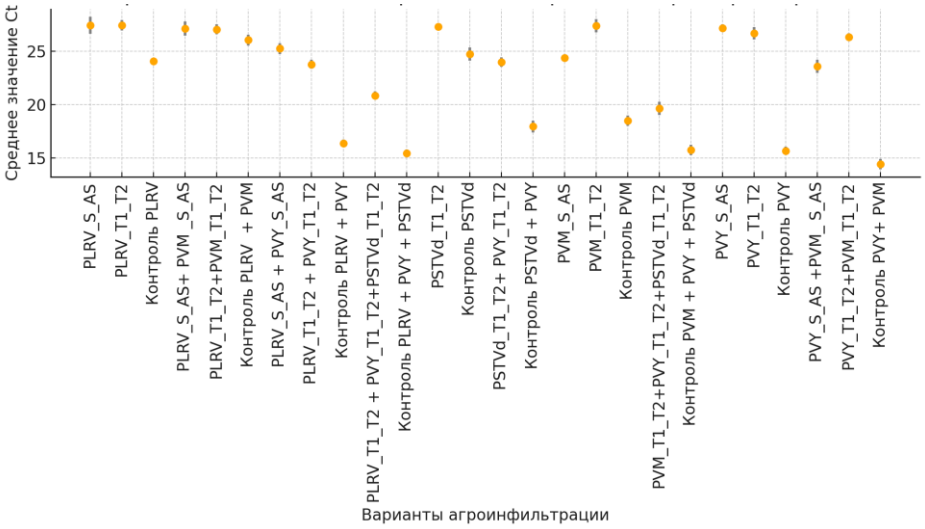


Figure 4. Amplification of Potato Viruses in Plants Expressing gRNA, Sense, and Antisense Sequences

Statistical Analysis and Construct Validation

Statistical analysis in R confirmed that all of the constructs tested led to significantly reduced viral loads as compared to untreated controls ($p < 0.05$). Single pathogen-specific constructs showed, while multi-pathogen constructs provided comprehensive suppression with high reproducibility. In addition, paired t-test and other statistical tools were used, so that the experimental results were reliable.

Discussion

The results indicate the potential of CRISPR/Cas13 and RNAi technologies as efficient tools for controlling viral infection in potatoes. The rapid action, specificity, and scalability of CRISPR/Cas13 systems make them a promising alternative to traditional approaches. The combinations of RNAi and CRISPR/Cas13 strategies pave the way for the generation of transgene-free and sustainable solutions for potato crop protection.

Further research should focus on optimizing guide RNA design, improving the durability of these systems under field conditions, and scaling up for broader agricultural applications. Such progress may also help secure global food supplies by preventing losses from viral pathogens in potatoes and other staple crops [24-30].

Conclusion

RNAi and CRISPR/Cas13 systems are effective for the control of important potato viruses. The constructs greatly reduced viral RNA levels, improved disease symptoms, and took the first steps toward effective and long-term viral control. CRISPR/Cas13 exhibited faster action and greater specificity compared to RNAi, making it a promising tool for the development of transgene-free crop protection strategies. Future research should focus on optimizing these technologies for field applications, paving the way for enhanced resilience in potato production and improved global food security.

Author Contributions

D.G – concept and supervision of the work; **K.A., A.K. –** conducting the experiments; **K.A., R.K. –** discussion of the research results; **K.A., R.K. –** writing the text; **D.G. –** editing the text of the article.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Индукцияланған РНҚ интерференциясы және оның өсімдіктердегі картоп вирусының амплификациясына әсері

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Аңдатпа. Картоп дақылдарындағы вирустық аурулар, әсіресе картоптың Y вирусы (PVY), картоп жапырағының шиыршық вирусы (PLRV), картоптың M вирусы (PVM) және картоп шпинделінің түйнегі вироиды (PSTVd), бақылауға ерекше қиындық туғызады және әлемдік ауыл шаруашылығы

үшін маңызды мәселе болып табылады. Осы зерттеуде біз РНҚ-интерференциясы (RNAi) және CRISPR/Cas13 технологияларының вирустық титрлерді төмендету және патогенездің алдын алу қабілетін бағалаймыз. Картоп өсімдіктері жеке және біріктірілген вирустық қоздырғыштармен жұқтырылды, содан кейін вирустық РНҚ-ның RNAi және Cas13 арқылы деградациясы үшін gРНҚ, сенсорлық және антисенс тізбектерін қамтитын конструкциялармен өңделді. Вирустық РНҚ деңгейін өлшеу үшін сандық ПТР (qPCR) қолданылды, ал аурудың дамуы үш апта бойы бақыланды. Cas13 экспрессиясы флуоресцентті микроскопия және Western blot әдістерімен расталды. RNAi конструкцияларының нәтижелері PVY үшін вирустық РНҚ деңгейін 85%-ға, ал PLRV үшін 78%-ға дейін төмендетті. CRISPR/Cas13 негізіндегі конструкциялар одан да жоғары басу деңгейін көрсетті (кейбір емдеу нұсқаларында ≥90%) және симптомдардың айтарлықтай жеңілдеуімен ерекшеленді. Бірнеше мақсатты бағыттаған конструкциялардың синергиялық әсерлері вирустық жүктеменің ең үлкен төмендеуімен және ауру ауырлығының айтарлықтай азаюымен байқалды. Өңделген және бақылау топтарындағы өсімдіктер арасындағы айырмашылықтар статистикалық тұрғыдан маңызды болды ($p < 0,05$). Бұл зерттеу RNAi және CRISPR/Cas13 технологияларының картоптың вирустық инфекцияларға төзімділігін арттыра алатынын көрсетеді. Бұл әдістер ауруларды бақылауға арналған кеңінен қолдануға болатын, трансгенсіз тәсілді қамтамасыз етіп, тұрақты ауыл шаруашылығына және жаһандық азық-түлік қауіпсіздігіне үлес қосады.

Түйін сөздер: картоп вирусы, РНҚ-интерференциясы, CRISPR/Cas13, гендерді редакциялау, вирус басу, молекулалық құралдар, тұрақты ауыл шаруашылығы, трансгенсіз технология

Индуцированная РНК-интерференция и её влияние на амплификацию вируса картофеля в растениях

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Аннотация. Вирусные заболевания картофеля, особенно вирус Y картофеля (PVY), вирус скручивания листьев картофеля (PLRV), вирус M картофеля (PVM) и вироид веретеновидности клубней картофеля (PSTVd), крайне трудно контролировать, и они представляют собой серьезную проблему для мирового сельского хозяйства. В этом исследовании мы оцениваем противовирусный потенциал технологий РНК-интерференции (RNAi) и CRISPR/Cas13 в снижении вирусных титров и предотвращении патогенеза. Растения картофеля были инфицированы как отдельными, так и комбинированными вирусными патогенами, а затем обработаны конструкциями, содержащими гайдовую РНК (гРНК), сенсорные и антисмысловые последовательности, используемые как для RNAi, так и для деградации вирусной РНК, опосредованной Cas13. Количественная ПЦР (qPCR) использовалась для измерения уровней вирусной РНК, а прогрессирование заболевания наблюдалось в течение трех недель. Экспрессия Cas13 была подтверждена с помощью флуоресцентной микроскопии и вестерн-блоттинга. Результаты экспериментов с конструкциями RNAi показали снижение уровня вирусной РНК

до 85% для PVY и 78% для PLRV. Конструкции CRISPR/Cas13 продемонстрировали еще более высокие показатели подавления вирусов (≥90% в некоторых вариантах обработки) с заметным облегчением симптомов. Синергетический эффект конструкций, направленных на несколько мишеней, привел к максимальному снижению вирусной нагрузки и тяжести заболевания. Различия между обработанными и контрольными растениями были статистически значимыми ($p < 0,05$). Это исследование демонстрирует, что RNAi и CRISPR/Cas13 могут повысить устойчивость картофеля к вирусным инфекциям. Обеспечивая масштабируемый, трансгенез-необусловленный подход к контролю заболеваний, эти методы способствуют развитию устойчивого сельского хозяйства и обеспечению глобальной продовольственной безопасности.
Ключевые слова: вирус картофеля, РНК-интерференция, CRISPR/Cas13, редактирование генов, подавление вирусов, молекулярные инструменты, устойчивое сельское хозяйство, трансгеннезависимая технология

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