Investigation of Viromes of Solanaceous Weeds

Solanaceae családba tartozó gyomok viromjának vizsgálata

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Abstract: Within the scope of our research study, we delve into a comprehensive investigation of virome of solanaceous weeds at the edge of crop fields and natural habitats. For this pilot study leaves of symptomatic *Datura stramonium* and *Solanum nigrum* were collected at two different locations near Keszthely in 2022. High-throughput sequencing (HTS) of small RNAs and RNAs were conducted and the sequenced reads were analysed using bioinformatic methods. Out of the identified viruses we confirmed the presence of the cucumber mosaic virus (CMV), encompassing RNA1, RNA2, and RNA3 components, as well as broad bean wilt virus 1 (BBWV1) RNA2 using RT-PCR. HTS resulted infection with several other viruses which presence are currently under validation. This pilot study contributes to our understanding of the viral diversity within *Solanaceae* plants, shedding light on the role of these plants as virus reservoirs. The implications of these findings may help to develop strategies for virus management of both endemic and invasive plant populations.

Keywords: *Virus diagnostics; Solanaceae; endemic plants; invasive weeds; cucumber mosaic virus; broad bean wilt virus; PCR; RT-PCR; HTS; virus reservoir*

Összefoglalás: Kutatásunk során a szántóföldek szélén és természetes élőhelyeken található Solanaceae családba tartozó két gyomnövény faj viromját vizsgáltuk. Kísérleteinkhez 2022-ben Keszthely közelében két különböző helyszínen gyűjtöttünk levélmintát vírusfertőzés tüneteit mutató Datura stramonium és Solanum nigrum növényekről. Kis RNS-ek és RNS-ek nagy áteresztőképességű szekvenálását (HTS) végeztük el, és a szekvenált olvasatokat bioinformatikai módszerekkel elemeztük. Az azonosított vírusok közül RT-PCR segítségével igazoltuk az uborkamozaikvírus háromosztatú genomjának mindhárom RNS-ét, valamint a széles babhervadás vírus 1 (BBWV1) RNS2 jelenlétét. A HTS a minták több más vírussal való fertőzését is mutatta, amelyek jelenlétének igazolása jelenleg folyamatban van. Eredményeink hozzájárulnak a Solanaceae növényeket fertőző vírusok változatosságának azonosításához és megértéséhez, és rávilágítanak e növények vírusrezervoárként betöltött szerepére, így segíthetik az endemikus és invazív növénypopulációk vírusfertőzöttségének megelőzését szolgáló stratégiák kidolgozását.

Kulcsszavak*: vírusdiagnosztika; Solanaceae; endemikus növények; invazív gyomok; uborka mozaik virus; széles babsorvadás virus; PCR; RT-PCR; HTS; virus rezervoár*

1. Introduction

The Solanaceae family represents a cohesive group of dicotyledonous plants, encompassing a range of extensively cultivated crops. Within this family, various species have significant roles, whether as vital food sources, suppliers of bioactive compounds, or even as decorative ornamental plants (Gebhardt, 2016). Members of this botanical family, including the potato (Solanum tuberosum), tomato, pepper and tobacco, thrive in regions across the globe with temperate or tropical climates (Hančinský et al., 2020). These plants have become staples in households worldwide, gracing kitchens, gardens, and fields, and contributing to global agriculture and culinary traditions (Olmstead et al., 2008). In addition to the commonly cultivated crops, the Solanaceae family is also home to various medicinal plants known for their alkaloid production. Notable examples include deadly nightshade (Atropa belladonna), black henbane (Hyoscyamus niger), and jimson weed (Datura stramonium) (Hančinský et al., 2020). Over the last century, plants from the Solanaceae family have played a crucial role in genetic research, contributing significantly to advancements in our understanding of plant genetics (Gebhardt, 2016). Cultivated species from the Solanaceae family are frequently found thriving alongside their wild counterparts in the same ecosystems. In many cases, wild Solanaceae species are considered common weeds within these shared habitats. In diverse agricultural settings, solanaceous plants are subject to various infectious pathogens, including viruses (Hančinský et al., 2020). Plant viruses are responsible for widespread epidemics in significant crops, posing a significant challenge to global food security. Consequently, virologists have traditionally prioritized their research efforts on economically vital crops, sometimes overlooking nearby weeds and wild plant species (Wren et al., 2006). In the realm of virus research, exploring the role of weeds as potential reservoirs has become pivotal. Scientists delve into the intricate dynamics between weeds and viruses, unravelling how these plants may serve as silent carriers. Understanding weed-associated viruses is crucial for devising effective strategies to mitigate agricultural threats and protect global crops. Weeds can act as reservoirs for both viruses and the insects that transmit them. In such circumstances, the spread of viruses can be highly pronounced. (Duffus, 1971). In our previous study we have tested the presence of viruses in solanaceous weeds using serological methods (Takács et al, 2001; 2006). In this current work we carried investigated viromes of solanaceous weeds using high-throughput sequencing based metagenomic methods.

2. Materials and Methods

In the year 2022, a study was conducted in Keszthely, Hungary, focusing on solanaceous plants displaying symptomatic features. This investigation encompassed two distinct fields, denoted as Field I (*Datura stramonium, Solanum nigrum*) and Field II (*Solanum nigrum*).

Field I: comprised *Datura stramonium*, germinated in early August, and *Solanum nigrum*, germinated from seeds in May. These plants coexisted alongside horticultural crops, including pepper, eggplant, and tomato. A comprehensive sampling strategy was employed, where five samples were collected from each plant. Specifically, leaves were collected from *Datura stramonium*, while both leaves and flowery shoots were gathered from *Solanum nigrum*.

Field II: was exclusively dedicated to *Solanum nigrum*, which had been germinated in May. This field had a previous history of wheat cultivation and potato farming, resulting in diverse dicot species covering the terrain. Similar to Field I, a systematic approach was adopted, involving the collection of five samples per plant from *Solanum nigrum*, encompassing both leaves and flowery shoots.

From the collected plant material total nucleic acid was isolated. To streamline the investigation, a strategic approach to sample pooling was employed. Three distinct pools were created: one for Field I *Datura stramonium*, one for Field I *Solanum nigrum*, and one for Field II *Solanum nigrum*. Each pool combined the RNA extracts from the respective plants within its category, resulting in a comprehensive representation of the viral content within each field. The three pools were mixed to prepare a pool containing extracts originating from both fields and plants. The pooled RNA samples were subsequently subjected to the construction of small RNA libraries, and RNA sequencing (RNA-seq). High throughput sequencing on Illumina platform was ordered as a service. Sequenced reads were analysed using the Qiagen CLC Genomic Workbench. Reads were trimmed and used for contig assembly.

Virus diagnostics were performed by conducting a BLAST search of the assembled contigs, referencing all known plant-hosted viruses in the NCBI database. The results were ordered based on their lowest E-value. We also mapped reads (both redundant and non-redundant) to the reference genomes of the HTS identified viruses. Consensus sequences were generated and the coverage of viral genomes by sRNA or RNA reads were determined. To validate the results of HTS and directly confirm the presence of cucumber mosaic virus (CMV) and broad bean wilt virus 1 (BBWV1), RT-PCR was performed. After cDNA synthesis, we conducted an actin test to assess cDNA quality. The resultant cDNA served as a template for RT-PCR using virus-specific primers to confirm the presence of CMV (RNA 1, RNA 2, and RNA 3) and BBWV1 RNA 2.

3. Results and Discussion

As a result of the HTS we got 16,5 small RNA and 21,6 million RNA reads, Form these reads 132523 (RNAseq) and 3177 (sRNAseq) contigs were built, respectively (Table 1.).

Table 1. Statistics of the HTS results

Library code	Sequenced reads	Trimmed reads all (containing redundants)	Non-redundant reads	Number of contigs
SOL_KES_10	16253182	15972388	15766344	132523
255_KSOL_S17	21955551	21573415	3178957	3177

Annotation of the contigs using reference genomes of known plant infecting viruses during the BLAST analyses revealed the presence of several different viruses: CMV, BBWV1, tomato vein clearing virus (TVCV), tomato yellow virus (TVY), Chenopodium quinoa mitovirus (CHQM), pepper vein clearing virus (PVCV), potato leafroll virus (PLRV) and Solanum symptomless mottle virus (SSMV).

Table 2. Summary of the results of the bioinformatics analysis of sRNAseq and RNAseq

HTS method	Feature	CMV			BBWV1		TVCV	TYV	CHQM	PVCV	PLRV	SSM
		RNA1	RNA 2	RNA 3	RNA 1	RNA 2						
sRNAseq	Number of contigs	62	54	31	16	5	25	0	0	0	0	3
	Coverage of the genome	99%	99%	100%	67%	64%	80%	43%	48%	63%	40%	55%
RNAseq	Number of contigs	1	1	2	1	1	19	5	7	5	4	22
	Coverage of the genome	99%	99%	99%	98%	97%	89%	90%	92%	90%	90%	88%

While RNAseq detected TVY, CHQM, PVCV, and PLRV in the sample we did not find contigs built up from sRNA reads specific to them. However, we observed extensive coverage of these viruses, even where no contigs were detected. This observation suggests that these viruses may be present in the samples, but they did not induce that strong silencing signal. Further studies are needed to fully understand the possible consequences of this finding and to determine the presence and possible function of these viruses.

The result of the bioinformatic analysis tells us how many different viruses could be detected in the sequenced pools, but does not contain information about the infection is the different species and individuals. Validation of the CMV and BBWV1 was done by RT-PCR (Figure 1) not only in the sequenced mixed pool, but also in the pools corresponding to the fields and plant species. Once the pool containing the virus was identified through RT-PCR, in-depth investigations were conducted on individual plants within that field (Figure 1.)

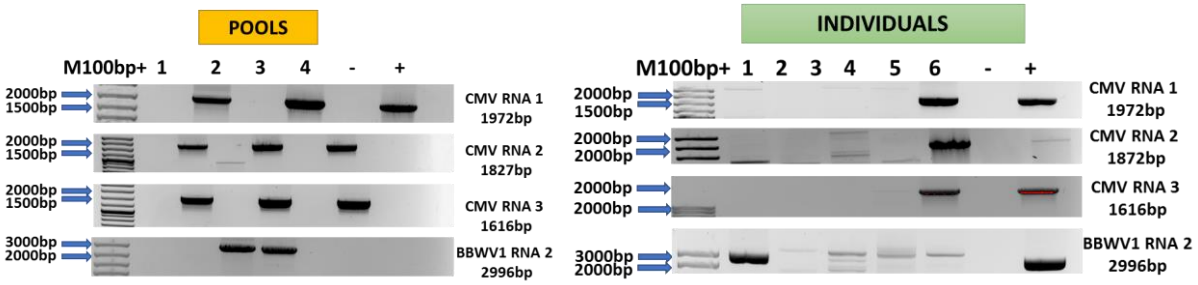

Figure 1. *Result of the RT-PCR analysis for testing the presence of CMV, and BBWV1 in Field I and Field II. M100bp+ is Thermo Scientific GenRuler 100bp+, 1 stand for pool Field I Datura stramonium, 2 stands for pool Field I Solanum nigrum, 3 stands for pool of Field II Solanum nigrum, while 4 is the pool of 1+2+3. - is MQ used as a negative control, while + was the positive control*

This methodology efficiently detected viruses in the selected fields, allowing us to focus on relevant subsets for a comprehensive analysis.

Notably, we observed infections with CMV and BBWV1, which were further confirmed by RT-PCR analysis. Both three RNAs of CMV could be detected in *S. nigrum* growing at field I. Testing the individuals growing at that field showed that only one plant (5) was infected with this virus. RNA2 of BBWV1 was detected in *S. nigrum* growing at field II, where one plant (1) was tested positive for its presence.

RT-PCR validation for the presence of the other viruses is currently ongoing.

4. Conclusions

During our work sRNA and RNA HTS showed their ability to reveal virus infection in the sampled weeds even when a pool of 15 plant extracts were sequenced. To reveal how frequent the coinfection of the plant with the presenting viruses we need further validation of the viruses in the individuals. The coexistence of multiple viruses in this small pilot survey highlights the complexity of the viral community within solanaceous plants and emphasizes the need for comprehensive monitoring and management strategies in agricultural settings. It is essential to continue studying the interplay between these viruses and their impact on crop health, as well as to develop targeted approaches to mitigate the potential negative effects on agricultural productivity if needed.

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