INVESTIGATION OF THE CAUSATIVE AGENT OF A VIRUS-LIKE SYMPTOM IN GRAPEVINE

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Abstract

Our group has been carrying on virus diagnostic surveys since 2013 using a special method: small RNA HTS. In 2018 strange symptoms, suggesting the presence of grapevine red blotch-associated virus (GRBaV), appeared at a Pinot Noir plantation. The virus was described in California in 2013 and its presence, except two descriptions (India and South-Korea) is confide to the North-American continent. To reveal the causative agent of the appeared symptoms we purified RNA from the symptomatic grapevine and made virus diagnostics by small RNA HTS. We confirmed the results using virus specific RT-PCR. Our results show that not the presence of the assumed virus cause the observed symptoms, moreover GRBaV is not present at the investigated vineyard. However, the plantation was infected with several other viruses. We

think that coexistence of several different viruses together with the inhomogeneity in the soil can both contribute in the symptom development, however to clarify this question further investigations would be needed.

Keywords: grapevine red blotch-associated virus, GLRaV-1, small RNA HTS, RT-PCR

Összefoglalás

Kutatócsoportunk 2013 óta végez vírusdiagnosztikai felméréseket szőlőültetvényekben egy új módszer, a kis RNS HTS használatával. 2018-ban egy Pinot noir ültetvényen furcsa, a szőlő vörös foltosodás vírus (GRBaV) jelenlétére utaló tüneteket figyeltek meg. A vírust 2013-ban Kaliforniában írták le, elterjedése az EPPO nyilvántartása szerint 2 kivételtől eltekintve (India és Dél-Korea) az észak-amerikai földrészre korlátozódik. A tünetek okainak felderítésére a jellemző tüneteket mutató szőlők RNS-éből kis RNS szekvenálással végeztünk vírus-diagnosztikát. A kapott eredményeket egy független módszerrel, RT-PCR-rel igazoltuk. Eredményeink azt mutatják, hogy a sajátos tünetek kialakulásában nem a feltételezett vírus játszott szerepet, a GRBaV nincs jelen a vizsgált ültetvényen. Az ültetvény viszont több vírussal is fertőzött volt. A tünetek kialakulásának az oka ez esetben a különböző vírusok együttes fertőzésében, az ültetvény talajának inhomogenitásában keresendő, de pontos megállapításához további vizsgálatok szükségesek.

Kulcsszavak: szőlő vörös foltosodás vírus, GLRaV-1, kis RNS HTS, RT-PCR

Introduction

Grapevine is considered as one of the major fruit crops in the world based on hectares cultivated and economic value. It can be infected with several (more than 80 is described until now) viruses which presence can affect not only its growth, but the quality of important

characteristics (berry weight and colour and sugar content, etc.). Grapevine red blotch-associated virus (GRBaV) was described in California, from a vineyard showing red blotch disease using HTS of ds RNAs (Rwahnih et al., 2013) and was proved to be the causative agent of the disease later (Yepes et al., 2018). It is a member of the Geminiviridae family, having a single circular DNA genome and its presence was proved to have inferior effect on berry development (Blanco-Ulate et al., 2017). The disease symptoms in red varieties include reddening of regions within leaf blades, along with red veins and petioles and delayed fruit maturity. In white varieties, leaves may develop yellow or chlorotic that is similar to leafroll-diseased vines. Asymptomatic vines can remain productive, but they also harbour viruses and act as potential reservoirs for virus spread to susceptible vines. It is graft transmittable, it could originated from a wooded riparian area by a supposed new vector (Cieniewicz et al., 2017). Grapevine virologists highlight that symptoms can be very similar to leafroll disease, with an exception that in GRBaV infected plants margin of the leaf stay flat and instead of green, pink veins appear (Sudarshana et al., 2015). As small RNA HTS can detect the presence of DNA viruses (Pooggin, 2018), we used this method to reveal the causative agent of the observed, virus-like symptoms.

Materials and Method

At the beginning of June 2018, the above symptoms: red blotches and red veins appeared at a Pinot noir plantation at Somogy (South of the Balaton) (Figure 1/A). At the vineyard plants with leafroll symptoms also appeared (Figure 1/B), and these two markedly different symptoms was altered even within a row.

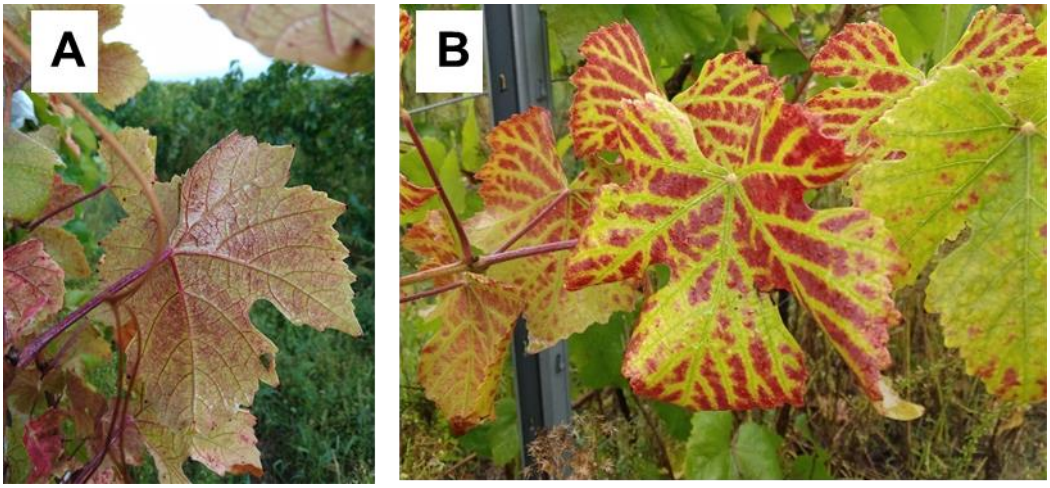

Figure 1. Pictures of the surveyed Pinot noir grapevines showing A/ red blotch-like, B/ leafroll-like symptoms.

Samples were collected from 4 individual plants of the same row, showing either red blotch-like (1, 3) or leafroll-like (2, 4) symptoms. We extracted RNA using a CTAB method and prepared two small RNA sequencing libraries (RB from plant 1 and 3 and LR from plant 2 and 4) according to our adapted protocol (Czotter et al., 2018a).

The sequenced reads were analysed using Qiagen CLC Genomic workbench. The reads were trimmed, both redundant and non-redundant list of sequences were prepared. This later one was used for contig building. Virus diagnostics were done by BLAST search of assembled contigs using all plant hosted viruses in the NCBI. The result list was ordered according to their lowest E-value. The reads (both redundant and non-redundant) were mapped to the GRBaV reference genome, and for other viruses which were present according to the analysis. Based on this analysis the consensus sequences were prepared and the coverage of the viral genome by small RNA reads were calculated. Threshold of virus presence was set to at least 1 virus specific contig, and higher than 60% coverage of the genome.

To validate the results of the small RNA HTS and directly test the presence of GRBaV, RT-PCR was carried out. After cDNA synthesis, an actin test was used to check the cDNA quality. This cDNA was used as a template to validate the presence of the presenting grapevine viruses

by RT-PCR using virus specific primers (for primer sequences please check (Czotter et al., 2018b). We also tested the cDNA with published GRBaV primers (Rwahnih et al., 2013).

Results and discussion

Results of the small RNA HTS show that several viruses: GLRaV-1, GVA, GFkV, GPGV and possibly GVB and viroids: HSVd, GYSVd-1 and 2 were present in the tested plants (Table 1), but neither GRBaV positive contig, nor reads mapped to the GRBaV genome were identified. Moreover, we could not get any product in the RT-PCR reaction using virus specific primers.

Table 1. Summary of the bioinformatics analysis together with the RT-PCR validation. GRBaV: grapevine red blotch-associated virus. Numbers indicate PCR positive samples out of the 2 which served for small RNA library preparation.

	type of analysis	viruses							viroids	
		GRBaV	**GLRaV-1**	**GVA**	**GVB**	**GFkV**	**GRSPaV**	**GPGV**	**HSVd**	**GYSVd-1/2**
RB library	small RNA HTS	0	+	+	0	0	0	+	+	+
	RT-PCR	0/2	1/2	1/2	0/2	1/2	2/2	2/2	2/2	2/2
LR library	small RNA HTS	0	+	+	?	+	0	+	+	+
	RT-PCR	No	2/2	2/2	0/2	2/2	2/2	2/2	2/2	2/2

GLRaV-1: grapevine leafroll associated virus-1, GVA: grapevine virus A, GVB: grapevine virus B, GFKV: grapevine fleck virus, GRSPaV: grapevine rupestris stem pitting-associated virus, GPGV: grapevine Pinot gris virus, HSVd: hop stunt viroid, GYSVd-1 and 2: grapevine yellow speckled viroid-1 and 2.

We have found severe infection with GLRaV-1 and also the presence of several other viruses and viroids (Table 1.) In case of GLRaV-1, GVA, GPGV and the viroids our RT-PCR results verified the result of the small RNA HTS (Figure 2). We could not prove the presence of GVB, but we have found only 1 GVB positive contig and less than 40% coverage of the genome, which indicate a false positive hit during the analysis. In RB library GFkV was not detected by

small RNA HTS, but in RT-PCR one of the plants showed infection. RNA from the other plant could dilute the sample what was used for small RNA sequencing, why we failed to detect it by this method. For GRSPaV we found the same contradiction, but this is what we usually experience in case of this virus. There were very few GRSPaV derived small RNA reads in the sample while the virus was present. One explanation of this can be that it was proved that this virus can have a positive effect on the grapevine physiology why defence mechanism against it could be suppressed during the evolution.

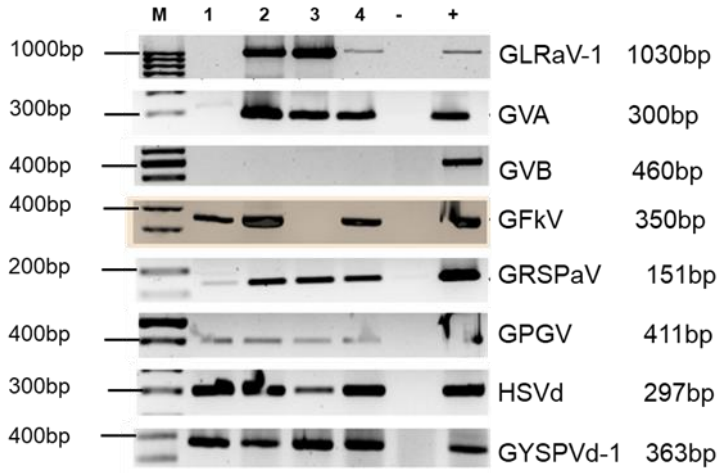

Figure 2. RT-PCR analysis for testing the presence of different viruses in the four plants which small RNA was sequenced.

In summary presence of several different viruses seemed random in the plants, thus we could not correlate any special combination with the appeared symptoms.

Conclusions

Our results showed that although red blotch symptoms appeared, GRBaV was not identified in the investigated Hungarian vineyard. Although it seemed possible for us to detect the presence of several different viruses and viroids, we cannot make any hypothesis about their contribution in the observed symptom development. Distribution of the nutrient in the soil of this vineyard

is very patchy why it is possible that shortage of some of them occurs quite randomly. Combination of these abiotic effects with the virus infections could lead finally or play role in the observed symptom development, but to find out the real causative agent we need further investigations. Moreover, these ambiguous results highlight the importance of the cooperation of classical and molecular virologist to reveal practical importance of the detected virus infections and explain or disclose possible causes of the emerging symptoms.

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