DETECTION OF VIRAL INFECTIONS IN A HUNGARIAN VINEYARD

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Abstract

Grape (*Vitis vinifera*) belonging to the family of *Vitaceae,* is one of the most important economic fruit crops in the world. For thousands of years in Hungry, grapevine has been cultivated in the Carpathian basin, where climatic and soil conditions are suitable for grape vine production. However, it is facing adversity of virus infections that influenced negatively the performance of the cultivars at an extent, which cause premature death of the stocks and generating great yield losses. The study was conducted in a Grapevine plantation from the area of Central Transdanubia (Lesencefalu) to diagnose important viral diseases that infect Othello variety and suggest measures possible for enhancing disease control and management strategies. Othello is also a commonly cultivated variety in Hungary with the assumption of possible tolerance to important grapevine diseases. 60 samples were collected from Othello grapevine variety and analysed in the laboratory to investigate virus infection using DAS-ELISA. Six viruses (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 and GFkV) were found

to infect this variety. Among those 60 samples, 27 samples were infected with viruses, almost half of the investigated leaf samples. The highest infection was found to be caused by GLRaV1 whilst the lowest infection was found to be associated with GLRaV3. However, 13 samples were found to be infected with more than one virus (multiple infection) whilst 14 samples were found to be infected with only one virus (single infection).

Keywords: DAS-ELISA, grapevine, virus, plant protection

Összefoglalás

A Vitaceae családba tartozó szőlő (*Vitis vinifera*) a világ egyik legfontosabb gazdasági gyümölcsnövénye. Magyarországon több ezer éve termesztenek szőlőt a Kárpát -medencében, ahol az éghajlati és talajviszonyok kedvezőek a szőlő termesztéséhez. A vírusfertőzések negatívan befolyásolták a fajták produktivitását és az állományok idő előtti leromlását és pusztulását okozzák. A Közép-dunántúli régió Lesencefalu szőlőültetvényein végeztünk vizsgálatokat, amelynek a célja a szőlőt fertőző fontos vírusos betegségek azonosítása, és a védekezési stratégia javítása volt. Az othello hazánkban is gyakori termesztésű fajta, feltételezve, hogy tolerálja a fontos szőlőbetegségeket. 60 mintát gyűjtöttünk Othello szőlőfajtából, és laboratóriumban DAS-ELISA segítségével azonosítottuk a kórokozókat. A vizsgálatok során hat vírus (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 és GFkV) jelenlétét azonosítottuk. A 60 Othello szőlőminta közül 27 volt fertőzött. A legnagyobb arányban a GLRaV1 jelenlétét igazoltuk, ezzel szemben a GLRaV3 előfordulása volt a legalacsonyabb. Összesen 13 minta esetében mutattunk ki komplex vírusfertőzést.

Kulcsszavak: DAS-ELISA, szőlő, vírus, növényvédelem

Introduction

Grape (*Vitis vinifera*) belonging to the family of *Vitaceae*, is one of the most important economic fruit crops in the world (Kumar, 2010). From Western Europe to the Persian shores of the Caspian Sea, the vine has demonstrated high levels of adaptability to most environments (Senthil *et al.*, 2011). The general classification of grapevines is largely divided into red- and white-berried cultivars dependent on their fruit skin colour, although other colours such as yellow, pink, crimson, dark blue and black-berried cultivars also exist. Red berried cultivars have anthocyanin pigments in berry skin, whilst white-fruited cultivars lack this pigment due to non-functional of regulatory genes of the anthocyanin biosynthetic pathway (Walker *et al*., 2007).

The fruit is processed into different products including juice, wine and raisins (dried fruit). It is also consumed as fresh (Buyukbay et al., 2011). Large percentage of grape production of the world is used to make wine (FAO, 2012). Grapes peel are essential source of oil and pectin. They also serve as raw material for production of cattle feed and used for preparation of candies (Kumar, 2010). Grapes are very useful in fighting diseases like dyspepsia, hemorrhoids, stones in the urinary tract and bile ducts. It also helps to activate the functions of the liver, support easy digestion, helps to reduce blood cholesterol level and eliminate uric acid (Kumar, 2010).

In 2012 according to FAO, the total world production of grapes stood at 51.42% with countries such as Spain, China, France, Italy, Turkey and Hungary contributing their quota in the production of grapes (FAO 2012; OIV, 2016). Spain cultivated with a vineyard area of 1038kha contributing to about 38.2mhL of wine production whilst Hungary cultivated 78kha which contributed to about 2.6mhL of wine production (OIV, 2016).

The first grape vine in Hungary was introduced by the Romans to Pannonia, and by the 5th century AD, there were records of extensive vineyards in what is now Hungary (Smithsonian,

2013). The main varieties in Hungary which are cultivated for white wine production includes; Furmint, Welschriesling, Bianca, Chardonnay, Cserszegi fűszeres and Rajna Riesling whilst red wine varieties are: Cabernet Franc, Blaufränkisch, Blauer Portugieser, Merlot and Zweigelt (Hajdu, 2018).

Viral diseases of grapes are common throughout all viticultural regions of the world. Infections resulting in damage to the vineyards causes a decrease in the yield and quality of grapes. Viruses disrupt various aspects of plant metabolism such photosynthesis, depress plant growth and development, transfer of respiration assimilants, reduce winter survival, drought resistance of the vine and its life cycle which directly affects the quality of wine, causing economic damage to the industry (Meng et al., 2017; Mannini and Digiaro 2017). Some important virus diseases that infect grapevines include Grapevine virus A (GVA), Grapevine virus B (GVB), Grapevine fanleaf virus (GFLV), grapevine fleck virus (GFkV) and grapevine leafroll-associated viruses. Leafroll is a damaging disease of the grapevine causing yield loss of up to 40% (Naidu et al., 2014).

Viruses are able to infect grapevines systemically. Their infection frequently is not promptly followed by symptom development that is, the pathogen remains latent for variable time intervals due to the low level of initial pathogen concentration in the host plant, to unfavourable environmental conditions or to the defense reactions of the host plant. Thus, these visually healthy, symptomless plants may carry the pathogens which are spread by vegetative propagating material. This phenomenon frequently triggers epidemic disease outbreaks in new plantations leading to significant economic losses and serious legal repercussions. Therefore, early and accurate diagnosis of plant virus diseases is a crucial component of all grapevine-management systems and also ensuring certification of propagating material as being pathogen-free are strongly regulated in most grape growing countries (Frison and Ikin 1991; OEPP/EPPO

2008; Rowhani et al. 2005). The aim of the experiment was to diagnose or test for the presence of most important grapevine infecting viruses so as to be able to make recommendations that would help improve disease management systems on Hungarian vineyards.

Material and method

The study was conducted in a grapevine plantation from the area of Central Transdanubia (Lesencefalu) (Figure 2). Lesencefalu is a small town with an area of 718 hectares in Tapolca District, Veszprém County, Hungary. It is situated 8 km north of Lake Balaton between Lesencetomaj and Várvölgy with Latitudes 46° 50′ 39.48″ N and Longitudes 17° 20′ 36.24″ E (Figure 1). This town takes its name from the Lesence stream that runs through it towards Lake Balaton.

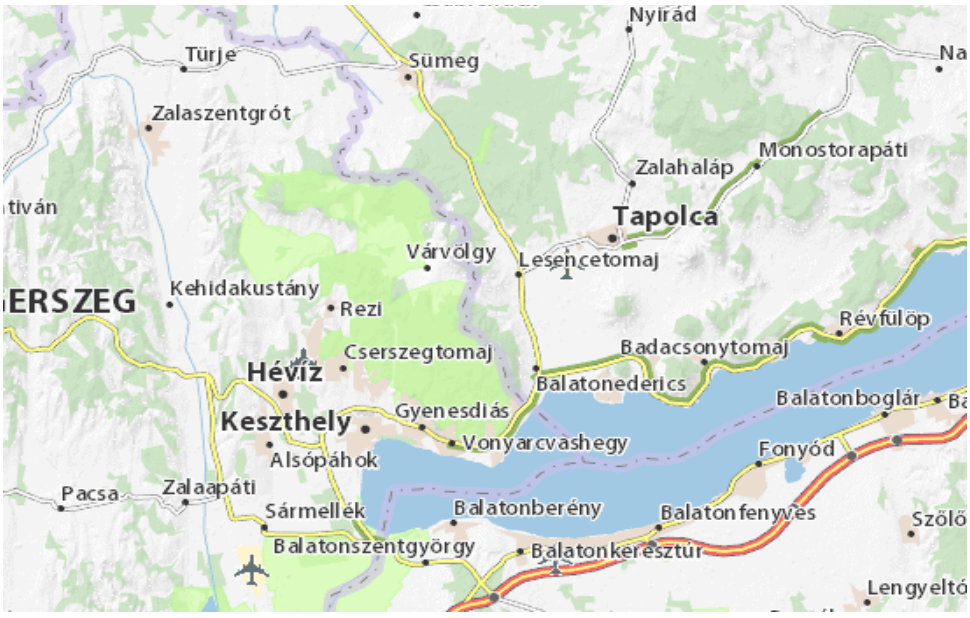

Figure 1: A map showing the study location (Google maps, 2021).

Figure 2. A map showing the exact study area (Google maps, 2021).

The samples were collected in July 2019 from one plantation (Lesencefalu) in the end of the summer. Othello is a widely cultivated variety in Hungary. It is also a direct growing cultivar with its cultivation mostly unsuitable. This is because during wine making, there is production of not only ethanol but also rising of methanol during fermentation process, making the variety not cultivated for wine making purposes. But there is an assumption that, because the Othello cultivar is not *Vitis vinifera* (*Vitis labrusca* x *Vitis riparia* x *Vitis vinifera*), it is tolerant or resistant against some of the main viral diseases of grapevine, hence virological studies about this variety is important. The number of samples collected were 60, and the plant part collected were the leaves. After collection, the samples were individually wrapped in polyethylene bags and stored at -20 C degrees until processed.

The samples were analysed or tested in the laboratory using serological Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS- ELISA) (Clark et al.,1976). The investigated viruses, Grapevine Leafroll associated virus 1 (GLRaV1), Grapevine Leafroll associated virus 2 (GLRaV2), Grapevine Leafroll associated virus 3 (GLRaV3), Grapevine Leafroll associated virus 6 (GLRaV6), Grapevine Leafroll associated virus 7 (GLRaV7) and

Grapevine fleck virus (GFkV) reagents were used from the LOEWE Biochemica. DAS-ELISA allows the reliable detection of the virus by using the available polyclonal antibodies prepared against the protein (Kritzman *et al*., 2001).

Figure 3. Preparation of experiments (Photo: György Pasztor).

Positive reactions are clearly visible, but we can get reliable results using an ELISA photometer (ELISA reader). The degree of color change was evaluated with a Labsystems Multiscan RC ELISA reader at 405 nm wavelength (Figure 3). We considered positive samples whose extinction values exceeded three times the negative control extinction value.

Results and discussion

Single virus infections are associated with one of the tested grapevine viruses infecting investigated samples. Among 60 samples, 27 (45%) samples were found to be positive/infected with at least one virus from 6 viruses (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 and Grapevine fleck virus (GFkV), whilst 33 (55%) samples were not infected with any virus

(showed negative virus infection). The magnitude of infection was different for each virus. 27 were positive/infected with GLRaV1, 12 were positive/infected with GLRaV2, 1 were positive/infected with GLRaV3, 5 were positive/infected with GLRaV6, 6 were positive/infected with GLRaV7 and 11 were positive/infected with GFkV (Table 1). This illustrates that, the Othello grapevine variety was found to be more susceptible to GLRaV1, the magnitude of infection was high 45% followed by GLRaV2 20%, GFkV 18.33%, GLRaV7 10%, GLRaV6 8.33% and GLRaV3 1.67% (Table 1). Only one sample was found to be infected with GLRaV3 indicating that, Othello grapevine variety was resistant/ not susceptible to GLRaV3. The highest infection numbers associated with GLRaV1 is in line with Martelli, 2014 and Habili et al., 2007 who reported that, GLRaV1 is one of the most widely spread and economically important viruses that infect grapevines. The virus can be found frequently in grapevines singly or in coinfection with other grapevine viruses.

The magnitude of infection associated with GLRaV1 was found to be high, followed with GLRaV2 and GFkV, this is because GLRaV1 is the most common virus in Hungary infecting grape vine, followed by GLRaV2 and GFkV. These viruses are the most common in Hungary which infect grapevine and cause great losses of yield and quality. These grapevine diseases are recognised as important viral diseases across grapevine-growing regions, and they are known to cause a wide range of negative impacts including overall resulting effects on vine performance, significant reduction in fruit yield and wine quality (Alabi et al., 2016; Lázár, 2003).

Table 1. Single virus infections of investigated Othello grapevine

TYPES OF VIRUSES	GLRaV1	GLRaV2	GLRaV3	GLRaV6	GLRaV7	GFKV
NUMBER OF INFECTED SAMPLES	27	12	1	5	6	11
PERCENTAGE (%)	45	20	1.67	8.33	10	18.33

For multiple infections, the results obtained from the research indicated that, 13 samples were found to be infected with more than one virus. Some samples were detected to be infected with either 2 or 3, 4, 5 viruses and other sample was found to be infected with all the 6 viruses (Figure 4). The result shows that 3 samples were infected with 2 viruses (1 sample was detected to be infected with both GLRaV1 and GFkV, whilst 2 samples were found to be infected both with GLRaV1 and GLRaV2). Also 3 samples were diagnosed to be infected with 3 viruses (GLRaV1, GLRaV2 and GFkV) (Figure 4). Likewise, the result demonstrates that 3 samples were infected with 4 viruses (2 samples were infected with GLRaV1, GLRaV2, GLRaV7 and GFkV whilst 1 sample was infected with GLRaV1, GLRaV2, GLRaV6 and GFkV). Similarly, 3 samples were diagnosed to be infected with 5 viruses (GLRaV1, GLRaV2, GLRaV6, GLRaV7 and GFkV) (Figure 4). However, only 1 sample was detected to be positive/infected with all the six viruses (GLRaV1, GLRaV2, GLRaV3, GLRaV6, GLRaV7 and GFkV). The result reiterates an occurrence reported by Al Rwahnih et al., 2009 and Seguin et al., 2014 who stated that, multiple virus infections are common in grapevine. Interestingly, GFkV was recorded in most multiple infection occurrences. The causal agent of fleck is widespread in grapevines. GFkV mostly exhibit symptomless infection in grapevine cultivars and is very often found in mixed infection with other harmful viruses such has GLRaVs, GVA, etc making it very difficult to singly point out its specific impact. Fleck seems to have a major effect on rootstocks, reducing growth and propagation attitudes (Credi, 2001). Indeed, the challenge and

target of future research should be geared towards the design of dependable strategies for preventing a quick sanitary deterioration of vineyards planted with costly certified materials. The realization of this goal would be largely dependent upon an integrated approach which involves a deeper understanding of the mechanisms for the replication of important viruses, their pathogenesis, interactions between these viruses and the grapevine host, as well as the interactions among different viruses in mixed infections (Maliogka et al., 2015).

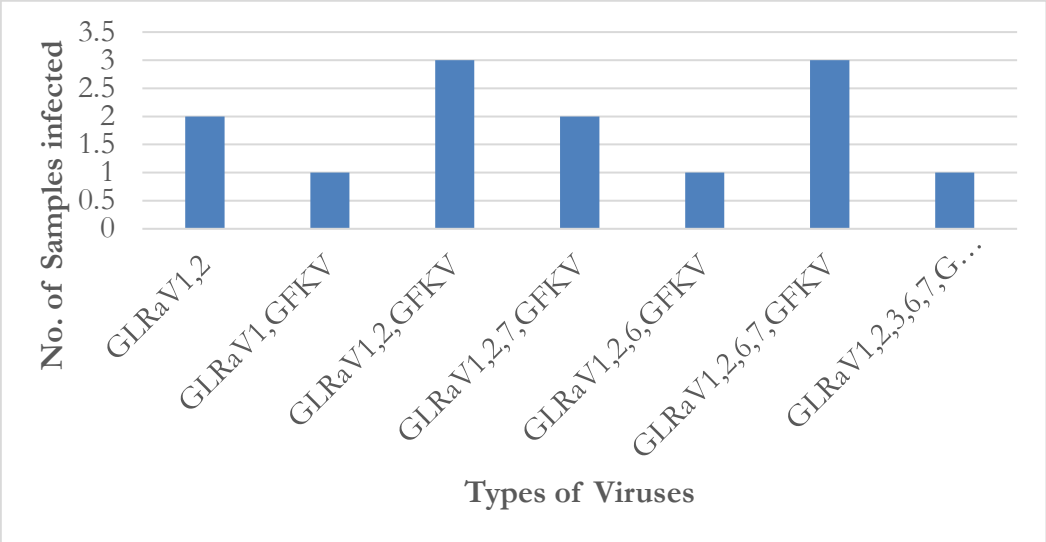

Figure 4. The Graph of Multiple virus Infection of Othello grapevine.

Conclusion

Grapevine has been operated for the purpose of propagation material, table grape and wine grape. Considering these importance and other benefits associated with its cultivation, and the fact that, viral diseases contribute to significant yield losses, studies continue to expand to enable us unravel the situation and intensify the methods of control and detection of viral diseases. This would help to limit the dissemination of viral pathogens by infected propagation and grafting materials as it is the most common way of virus spreading.

After testing the presence of infection by 6 viruses of the Othello variety by using DAS- ELISA, the results have demonstrated that, this variety is prone to important grapevine diseases more especially GLRaV1. For effective control and ultimately protection of grapevines against these diseases, early and frequent detection or diagnosis remains the initial driving force for revealing grapevine virus infestations and consequently the best control strategy. Moreover, the epidemiological characteristics of GLRaV1 appear to be similar to other *ampeloviruses* and integrated control strategies, involving a combination of virus-tested planting stock, cultural practices and vector control could be employed to minimize the spread of the viral diseases.

More studies could also be conducted to develop the immune resistant of the variety against those viruses. Breeders could select Othello grapevine variety as a breeding cultivar against GLRaV3, and it could be improved to be resistant/tolerant against other detected viruses. Furthermore, we need generic application which is improved in association with more precise and innovative techniques. However, Next generation sequencing is important for deepening the knowledge on the infection mechanism of the different viruses and the immune system response of the host. This will open new perspectives in the diagnostic field to detect the presence of viruses and identify other viruses which may have not been described yet in Hungary.

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