General properties of grapevine viruses occurring in Hungary

Magyarországon előforduló szőlővírusok és a hazai szőlő vírusbetegségek áttekintése

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Abstract

The past fifty years important advances have been made in the field of grapevine virus research, including characterization of pathogens and control measurements. Still the occurrence of *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Tomato black ring virus* (TBRV), *Grapevine chrome mosaic virus* (GCMV), *Alfalfa mosaic virus* (AMV), *Grapevine Bulgarian latent virus* (GBLV), *Grapevine fleck virus* (GFkV), *Grapevine leafroll- associated viruses* (GLRaV1-4), *Grapevine virus A* (GVA), *Grapevine virus B* (GVB) and *Grapevine rupestris stem pitting- associated virus* (GRSPaV) have been reported in Hungary and characterized by conventional methods as woody indexing, herbaceous indexing and serological methods. Among grapevine viruses the Grapevine line pattern virus (GLPV) seems to be uncial; because it was reported only in Hungary. Causal agents of several grapevine diseases, like enation, vein necrosis and vein mosaic remained undiscovered. These virus-like diseases occurred only sporadically, without economic importance.

Key words*:* certification programme, grapevine, viruses

Összefoglalás

Magyarországon az elmúlt ötven évben kezdődtek meg vizsgálatok a szőlővírusok kutatása területén, úgymint a kórokozók jellemzése és a szőlővírusok kimutatására, valamint a fertőzött tőkék kiszűrésére szolgáló azonosítási rendszer kiépítése.
Ez idő alatt Magyarországon hagyományos módszerekkel (tünettani megfigyelés, lágyszárú tesztelés, fásszárú tesztelés, szerológiai vizsgálatok) a szőlő páfránylevelűség vírust (*Grapevine fanleaf virus*, GFLV), az arabisz mozaik vírust (*Arabis mosaic virus*, ArMV), a paradicsom gyűrűs foltosság virust (*Tomato blackring virus*, TBRV), a szőlő krómmozaik vírust (*Grapevine chrome mosaic virus*, GCMV) és a szőlő bulgáriai látens foltosság vírust (*Grapevine bulgarian latent virus*, GBLV) mutatták ki a *Nepovirus* nemzetség tagjai közül. Azonosították továbbá a lucerna mozaik vírust (*Alfalfa mosaic virus*, AMV), a szőlő látens foltosság vírust (*Grapevine fleck virus*, GFkV), a szőlő levélsodródását okozó vírusok 1-4. szerológiai csoportjait (*Grapevine leafroll associated virus 1-4*, GLRaV 1-4), a szőlő A vírusát (*Grapevine virus A,* GVA), a szőlő B vírusát (*Grapevine virus* B, GVB), és a szőlő rupestris

faszöveti barázdáltság vírusát (*Grapevine rupestris stem pitting associated virus*, GRSPaV).
A hazánkban ez idáig leírt kórokozók közül a szőlő vonalas mintázottság vírusa (Grapevine line pattern virus, GLPV) az egyetlen, amelyet csak Magyarországon írtak le. Megfigyeltek tovább olyan feltehetőleg vírusos eredetű megbetegedéseket, amelyek kórokozóját még nem sikerült pontosan azonosítani. Ezek a szőlő enációja, az érnekrózis és az érmenti mozaik. Az utóbbi három betegségnek a fellépése szórványos és gazdasági jelenősége nincs.

Kulcsszavak: certifikációs program, szőlő, vírusok

Introduction

Because of continuous vegetative propagation, grape plantations are permanent targets of different viruses. The results of constant infections are: reduced yield and quality, shortening in productive period, weakening in rooting of propagation materials, reduction in disease resistance to abiotic and biotic stressors, and at least the early dieback of grape stocks. In Hungary much research emphasis has given to detect virus diseases of grapes and use virus-free stocks. In the past fifty years first pioneering studies have made by Dr. János Lehoczky and his co-workers in the Research Institute for Viticulture, in Kecskemét (Lehoczky, 1965). However, in the past decades new viruses emerged, some of them were here all the time, maybe they were not detected, and some can get into the country with infected propagation material. The names and taxonomy of viruses basically altered, and the identification methods are also completely changed. To clear up the present situation the aim of study was to offer a brief survey on the occurrence of the grapevine viruses in Hungary.

Virus screening in Hungary

Regular virus screening of grapevine varieties using ELISA tests and internationally accepted indicator species started in 1972 (Lázár, 1996 and 1998; Lázár *et al*., 2002). In these program the first year, symptom less grapevine stocks were selected and marked twice in the vegetation period, first at the flowering and then in the second half of September. At the time of the first selection, samples were performed for ELISA. Since 1985 ELISA has been routinely applied for detection of 7 viruses: *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Tomato black ring virus* (TBRV), *Grapevine chrome mosaic virus* (GCMV), *Grapevine leafroll-associated virus* 1, -3 (GLRaV-1, -3) and *Alfalfa mosaic virus* (AMV). Canes of symptom less and ELISA-negative plants were collected for further investigations and stored in plastic bags at 2-3^{o}C in cold room. In the spring of the second year, over wintered canes were checked by woody indexing on eight indicator species in the field: namely FS-4, *Vitis rupestris* cv. St George, *Vitis vinifera* cvs. Pinot noir and Chardonnay, *V. berlandieri* x *V. riparia* Kober 5 BB, *Couderc* x *V. berlandieri* LN 33, *V. riparia* Gloire, *V. rupestris* x *V. berlandieri* 110 R. Symptoms on woody indicator plants were recorded in June and in September. In the spring of the second year the over wintered canes occasionally checked also by mechanical transmission onto herbaceous indicator plants: *Chenopodium quinoa*, *C. amaranthicolor*, *Cucumis sativus* cv. “Delicates”, *Gomphrena globosa*, *Nicotiana clevelandii*, *N. tabacum* cv. “Samsun”, *N. glutinosa*, *Phaseolus vulgaris* cv. “Beautiful”. In the third and forth years

the nursery was evaluated twice. At the end, the marked grapevine plants, giving negative results on all indicators were considered virus-free (Lázár *et al*., 2002). The majority of viruses were detectable by ELISA tests, but not all. For example against Rupestris stem pitting associated virus no antiserum was commercially available. In these cases it was necessary to use visual selection and biological indexing.

Virus infection was surveyed in Hungary in the last three years. Occurrence of ten viruses was tested by DAS-ELISA serological methods in grape plantations.

The presence of viruses was checked for Grapevine fanleaf virus (GFLV), Arabis mosaic virus (ArMV), Tomato black ring virus (TBRV), Grapevine chrome mosaic virus (GCMV) for Grapevine leafroll-associated virus 1-3, (GLRaV 1-3), Grapevine virus A (GVA), Alfalfa mosaic virus (AMV) and Grapevine fleck virus (GFkV) using different specific antisera. Sixteen samples proved to be infected by GLRaV-1, one by GLRaV-2, and sixteen by GLRaV-3. GCMV were found in eight cases. ArMV caused but only in seven cases. GFLV occurred five times, TBRV occurred in five samples. The twenty six GFkV infected samples originated from the North part of Hungary from Szekszard. No GVA infected plants have been found among the samples (Cseh *et al*., 2011).

Recently, molecular tests have been developed that directly target the genetic material (genome) of plant pathogens (Weber *et al*., 2002). The most sensitive methods are molecular tests for pathogen detection. The common rapid method was among them the RT-PCR. Commercial PCR testing is currently available for many viral pathogens of grapevines, but it is an expensive and time consuming method, so it is suggested just in extreme cases only. Still no grapevine viruses have been characterized by molecular methods in Hungary. The potential for using such procedures for routine diagnosis of grapevine viruses and virus-like diseases offers new opportunity for understanding the disease complexes and possibility controlling the diseases.

Virus disease groups

Grapevine viruses or virus like agents has been characterized on the basis of most characteristic symptoms as: a) degeneration, b) leaf roll, c) fleck, d) rugose wood, e) yellow mottle, f) line pattern, g) enation, h) vein necrosis or i) vein mosaic (Cseh *et al.,* 2008).

Grapevine degeneration

Degeneration is a general expression of grape decline caused by different Nepoviruses that was found in Hungary: *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Grapevine chrome mosaic virus* (GCMV), *Grapevine Bulgarian latent virus* (GBLV) and *Tomato black ring virus* (TBRV). After the newest classification the GFLV, ArMV, GCMV, TBRV and GBLV belong to *Secoviridae* family, *Comovirinae* subfamily and *Nepovirus* genus (Carstens, 2010).

This bulk of viruses cause great crop losses and lower fruit quality and are widespread throughout the vineyards of the world. Sometimes they cause heavy yield losses, up to 80% of the crop (Martelli, 1993; Pompe-Novak *et al*., 2007). However, a few of them cause great losses locally but because their limited geographic distribution have limited economic importance. Some of these viruses are widespread

but there are mildly pathogenic or cause few or no symptoms in most of the grapevine cultivars.
Nepoviruses have polyhedral virions with a diameter of about 25- 30 nm. Their genomes consist of two single-stranded RNAs in two components. The nematode vectors acquire the virus by feeding on roots of diseased plants and can retain it for some months.
The disease spreads locally from grapevine to grapevine often in somewhat circular areas. Vectors may spread with rooting from nurseries and soil by water from flooding. Many Nepoviruses may be transmitted to weed hosts and some are seed-borne in weeds. Long distance spread of Nepoviruses occurs in the transfer of grapevine rooting, cuttings and graft wood (Brunt *et al*., 1996).
Unfortunately limited information is available on the molecular determinants involved in the transmission process of nepoviruses. Previous studies indicated that the viral determinants responsible for the specificity of nepovirus transmission map to RNA2 coat protein (Andret-Link et al., 2004).
Detection of these viruses largely based on symptoms produced either on the cultivar itself or on another more sensitive cultivar or *Vitis* species as indicator plants transmitted by grafting. By the woody indexing susceptible cultivars as FS-4 201- 39 (Siegfriedrebe), *Vitis rupestris* cv. St. George, *Vitis vinifera* cv. Chardonnay used as indicators (Kölber *et al*., 1981). Some viruses can be transmitted mechanically to herbaceous host plants as *Chenopodium quinoa, C. amaranthicolor, Cucumis sativus* and *Gomphrena globosa.* In Hungary described nepoviruses can be relative easily identified by ELISA tests, using extracts of infected grapevine tissues.

Grapevine fanleaf virus (GFLV)

The virus occurs in all viticulture areas of the world. In Hungary it was detected first by Sárospataki in 1964. Symptoms vary in type and severity according to the strains present in the plant. The infection can cause a quick destruction of the plant or cause a decline over several years. This is the most important viral pathogen of grapevine plantations, infecting all the cultivars. Infection affects both: the productivity and the longevity of grapevines (Andret-Link *et al*., 2004b).
The symptoms of infectious malformation virus strain (Martelli and Boudon-Padieu, 2006) are double nodes, short internodes on the infected grapevines, abnormal brunching and fasciations, leaf deformation of various types, enlarged petiolar sinus, primary veins gathered having the shape of a fan. Leaf blade is often asymmetrical, with acute denticulations, irregular veins and various patterns of chlorotic mottles, yellow mosaic and vein banding. The brunches of the infected plants are smaller and fewer. The berries very often fail to develop (dropping off) or remain small and seedless (Martelli, 1993).
GFLV have two in Hungarian literature described strains: the yellow mosaic (GFLV-YM) and the vein banding (GFLV-VB) strains (Lázár, 1996). The symptoms of these strains were observed in Hungary by Lehoczky in 1965. By the yellow mosaic induced chromogenic strains affected vines show chrome-yellow discolorations that develop early in the spring and may affect all vegetative parts forming irregular rings and lines, variously extended mottling of the veinal or interveinal areas, to total yellowing (Martelli, 1993). Vein banding is characterized by yellow mottling or chrome yellow bands along the principal veins of particularly during summer and fall. Leaf symptoms on rootstock varieties are blotch mottling, line pattern, and veinlet clearing (Goheen and Hewitt, 1962). Recently suggested vein banding symptoms to be

caused by a co-infection of a viroid (*Grapevine yellow speckle viroid*) and GFLV (Martelli and Boudon-Padieu, 2006).

GFLV virus particles are isometric and about 30nm in diameter. The viral genome is composed of two single-stranded positive-sense RNAs (Andret-Link *et al*., 2004a). It is vectored by *Xiphinema index* by noncirculative and semi-persistent manner (Demangeat *et al*., 2005). *Xiphinema italie* has also been reported to be a vector, but never been confirmed by other investigations (Andret-Link *et al*., 2004). On *Chenopodium quinoa,* GFLV infection cause chlorotic spots and vein banding, systemic mottling and deformations (Bashir *et al*., 2007).

Arabis mosaic virus (ArMV)

First ArMV was reported from grapevines in the former Yugoslavia (Panjan and Saric, 1963). In Hungary was found in 1966 by Martelli and Lehoczky (Martelli and Boudon-Padieu, 2006). ArMV serologically related to GFLV. Symptoms are similar to those of fanleaf, and mixed infections with fanleaf may occur. ArMV has a wide natural host range (Wetzel *et al*. 2004).Vectors species are: *Xiphinema diversicaudatum, Xiphinema coxi* and *Longidorus caespiticola,* but spreads by seeds and propagation material as well. ArMV infection may also induce striking chlorotic ring spots in *Nicotiana glutinosa* (Martelli, 1993).

Tomato black ring virus (TBRV)

This virus was first described in 1963 by Stellmach and Bercks (Martelli and Boudon-Padieu, 2006). In Hungary Lehoczky and Burgyán isolated it first in 1986 (Lehoczky and Burgyán, 1986). Infection produces a reduction in growth, a mottling of older leaves and yellowing of the edges of the leaf blade. In early stage of infection the leaves show chlorotic spots, rings and lines. A high percentage of graft failure is common. The spread of TBRV occurs by seeds, grafting and the longidorid nematode *Longidorus attenuatus* (Jończyk *at al*., 2004).

Grapevine chrome mosaic virus (GCMV)

GCMV was firstly isolated in Hungary in the world, near the Lake Balaton by Martelli, Lehoczky and their co-workers (Martelli, 1966; Lehoczky *et al*., 1984b). The symptoms include chrome-yellow to whitish discolorations of the leaves, lack vigour, unfruitfulness, short internodes or double nodes. Some strains induce malformations and chlorotic mottling of the leaves or remain symptomless. The vector is not known for certain, but the disease appears to be soil-borne. Systemic hosts of GCMV are: *Chenopodium quinoa, Nicotiana occidentalis, N. clevelandii* and *Cucumis sativus* (Lehoczky *et al*., 1984b; Le Gall *et al*., 1997).

Grapevine Bulgarian latent virus (GBLV)

GBLV was first described formerly by Martelli *et al.,* in 1977. In Hungary, Pocsai found first (Pocsai, 1981). GBLV infects several cultivars without symptoms and is transmissible to several herbaceous hosts by mechanical inoculation, for example *Chenopodium quinoa*. It can cause delayed bud break, irregular elongation of the shoots, straggly fruit cluster, reduced growth and fanleaf-like symptoms. The specific

vector is not known, but with seed and propagation material can spread (Martelli, 1993).

Grapevine fleck

The agent of fleck disease is *Grapevine fleck virus* (GFkV). It was described first in California by Hewitt *et al*. in 1962. In Hungary Lehoczky was found first in 1981 (Lehoczky and Farkas, 1981). GFkV is latent on *Vitis vinifera*, but indexing is effective on *V. rupestris* especially on cv. *St George* using classical grafting of woody cuttings (Schieber *et al*., 1997). The symptoms on test plants are clearing of the veins of third and fourth order producing localized translucent spots, which are best seen by holding the leaves against the light. Leaves with intense flecking are wrinkled and twisted and may curl upward. Its virions are isometric cca. 30 nm in diameter, have a rounded contour and a surface structure. The viral genom is positive-sense, single-stranded RNA. GFkV is the type member of the genus *Maculavirus* in the family *Tymoviridae* (Abou Ghanem-Sabanadzovic *et al*., 2003). The virus is non-mechanically transmissible phloem-limited. The disease can experimentally transmit through dodder, and can spread through propagation material (Martelli, 1993; Martelli *et al*., 2002).

Grapevine leafroll

Grapevine leafroll symptoms maybe induced by a complex of viruses, the majority of which belong to the *Closteroviridae* family. Nowadays, nine serologically distinct viruses, designed Grapevine leafroll-associated viruses (GLRaV 1-9), have been associated with the disease complex (Meng *et al*., 2005).
The disease cause yield losses of as much as 20 to 40% (Routh *et al*., 1998). The leafroll symptoms were described first in Italy by Sannino in 1906 (Sannino, 1906; Martelli and Boudon-Padieu, 2006). It was reported first by Lehoczky and co-workers in 1969. In Hungary the GLRaV 1-4 viruses have been found (Lázár *et al*., 1995a). Grapevine leafroll-associated virus 7 was also noticed in Hungary (Choueiri *et al*., 1996). Leafroll disease causes reduced sugar content of fruit, delayed fruit maturity, abnormal discoloration of the fully expanded leaves and downward curling of leaves (Little and Rezaian, 2006). The symptoms have seen mainly at the end of growing season (Meng *et al*., 2005).
A new genus: *Ampelovirus*, including *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 3* (GLRaV-3) with *Grapevine leafroll-associated virus 5* (GLRaV-5), was proposed distinct from the genus *Closterovirus* (Komínek *et al*., 2005). Tentative species of the genus *Ampelovirus* are: Grapevine leafroll- associated virus 4 (GLRaV-4), Grapevine leafroll-associated virus 6 (GLRaV-6), Grapevine leafroll-associated virus 8 (GLRaV-8) too. However, the *Grapevine leafroll-associated virus 2 (*GLRaV-2*)* belongs to the genus *Closterovirus*. Virions of *Closteroviridae* family, included the *Closterovirus* and *Ampelovirus* genera, are filamentous elongated particles (ca. 2000 nm), and consist a positive-sense RNA genome (Brunt *et al*., 1996). Grapevine leafroll-associated virus 7 (GLRaV-7) is presently classified as unassigned species to the family.
Grapevine leafroll-associated virus 1 (GLRaV-1) spreads via propagation material and grafting and to be transmitted by the scale insect *Parthenolecanium corni, Pulvinaria vitis, Neopulvinaria innumerabilis* and by mealybugs *Heliococcus bohemicus* and *Phenacoccus aceris* (Komínek *et al*., 2005, Martelli and Boudon

Padieu, 2006). In Hungary the last two species occur on grapevine (Jakab and Szendrey, 1989).
Grapevine leafroll-associated virus 3 can be transmitted by *Planococcus citri* too, with non propagative mechanism (Cid *et al*., 2007). Another insects has been assumed were found to be vectors the viruses, as *Planococcus ficus*, *Pseudococcus longispinosus*, and other *Pseudococcus spp* (Cabaleiro and Segura, 2006) and has been reported to be vectored by soft scales, such as *Pulvinaria vitis* and *Neopulvinaria innumerabilis* (Sforza *et al*., 2003).
The natural vector of GLRaV-2 is still not known, the main transmission route seems to be by vegetative propagation and grafting. Its herbaceous hosts are *Nicotiana benthamiana* and *N. clevelandii* (Beuve *et al*., 2007). By the wood indexing *Vitis vinifera* cultivars can be use, especially red fruited varieties as: Pinot noir, Cabernet franc and Merlot (Martelli, 1993).
Grapevine leafroll-associated virus 5 and *Grapevine leafroll-associated virus 9* are also transmitted by *Pseudococcus longispinosus* (Golino *et al*., 2002; Martelli and Boudon-Padieu, 2006).
On the International Committee on Taxonomy of Viruses (ICTV) modified list can be found just the GLRaV-1,-2,-3 and -5 groups from the earlier 9 (Carstens, 2010).

Rugose wood complex

Rugose wood is a complex of graft transmissible diseases of grapevine that occur worldwide. The first description of the symptoms has been made by Hewitt in 1954 in California, as a virus-like disease “rough bark” (Hewitt, 1954). In Hungary it was detected by Martelli *et al*. (1967).
Symptoms are pitting and grooving in the woody cylinder. These diseases are responsible for graft incompatibility, delayed budburst, severe decline and even death of vines. Four disorders are associated with rugose wood: Rupestris stem pitting, Kober 5BB stem grooving, LN 33 stem grooving and grapevine corky bark (Lázár *et al*., 1995b). The aetiology is not completely clarified, but several evidence suggest that *Grapevine virus A* (GVA), *Grapevine virus B* (GVB) and *Grapevine rupestris stem pitting associated virus* (GRSPaV) are involved in rugose wood.
In 2009, the International Committee on Taxonomy of Viruses (ICTV) modified the list and the classification of the viruses. A new family *Betaflexiviridae* (Carstens, 2010) includes the rugose wood complex caused viruses GVA, GVB and GRSPaV.
Rupestris stem pitting disorder can be associated with GRSPaV. On the trunks of indicator variety strips of small pits on the woody cylinder develop mainly below the site where the inoculum is grafted (Zhang *et al*., 1998; Meng *et al*., 1999). Chronic infections produce stunting (Lima *et al*., 2006).
GRSPaV belongs to genus *Foveavirus* (Nolasco *et al*., 2006) with single stranded RNA genom and filamentous, flexuous capsid with length of 800 nm (Brunt *et al*., 1996). The virus is not mechanical transmissible and no natural vector has found until now (Nakaune *et al*., 2008). It can be detected on *Vitis rupestris* cv. St George.
Grapevine virus A (GVA) involved in the aetiology of Kober stem grooving. GVA is phloem-limited virus with filamentous, flexuous particles about 800 nm long with single-stranded RNA genom. GVA and GVB belong to the genus *Vitivirus* (Murolo *et al*., 2008).
GVA is transmitted by several species of the pseudococcid mealybug genera *Pseudococcus* and *Planococcus*, as *Pseudococcus longispinosus, P. affinis, Planococcus ficus, P. citri, Heliococcus bohemicus and* soft scales *Neopulvinaria*

innumerabilis and Parthenolecanium corni (Minafra *et al*., 1997, Hommay *et al.,* 2008). The virus has herbaceous hosts as *Nicotiana benthamiana, N. clevelandii* and *Gomphrena globosa* (Haviv *et al*., 2006). Indexing on *Vitis berlandieri x Vitis riparia* cv. Kober 5BB gives the best results (Lázár, 1996).
Grapevine virus B (GVB) has been recognised as the causal agent of corky bark. The genom structure and the virions are similar to the GVA. Corky bark shows symptoms on *Vitis rupestris* cv. St George and *Vitis berlandieri x Couderc 1613* cv. LN 33.
GVB is transmitted by *Pseudococcus longispinosus* (Kuniyuki *et al*., 2006), *Ps. affinis* and *Planococus ficus* mealybugs also in a semipersistant manner (Martelli and Boudon-Padieu, 2006). Its herbaceous hosts are *Nicotiana benthamiana*, *N. clevelandii* and *N. occidentalis* (Saldarelli *et al*., 2005).
The causal agent of LN 33 stem grooving is still not known. Symptoms can see only on *Vitis berlandieri x Couderc 1613* cv. LN 33 (Lázár, 1996).

Grapevine yellow mottle

Yellow mottle caused by the infection of *Alfalfa mosaic virus* (AMV). The disease was first described in Germany by Bercks *et al*. in (1973) and in Hungary by Lehoczky and Beczner (1980). AMV is pathogen with wide host range. Infected grapevines show various patterns of yellow discolorations. The yellowing does not extend to the veins, remain green. In summer symptoms became whitish, but not masked. The virus has polymorphic particles with a tripartite genome. It has a positive sense, single stranded RNA. AMV is the type member of the genus *Alfamovirus* in the family *Bromoviridae* (Brunt *et al*., 1996). AMV transmitted mechanically, by grafting, by aphids and by the propagation material (Martelli, 1993). Herbaceous test plants are: *Chenopodium quinoa, Ch. amaranthicolor, Phaseolus vulgaris, Nicotiana tabacum, N. glutinosa* (Beczner and Lehoczky, 1980) and the woody indicator plants are: *Vitis vinifera* cv. Chardonnay, *V. vinifera* cv. Pinot noir, *V. rupestris cv.* St. George. (Lázár, 1996)

Grapevine line pattern

Line pattern symptom caused by Grapevine line pattern virus (GLPV). This disease is known to occur only in Hungary, it was described first in the world by Lehoczky *et al*. (1987). Infected leaves show bright discolorations forming marginal rings, scattered spots or blotches, or maple leaf like line patterns. The causal agent is a putative member of the genus *Ilarvirus* in the family *Bromoviridae*. This virus has polymorphic particles and multipartite RNA genome. It consists of single-stranded RNAs (Brunt *et al*., 1996). Herbaceous tests plants are: *Chenopodium quinoa, C. amaranthicolor, Phaseolus vulgaris, Nicotiana glutinosa, Cucumis sativus* etc. The best grape indicator is *Vitis vinifera* cv. Jubileum ’75 (Lehoczky *et al*., 1987).

Grapevine enation

Causal agent of enation disease is still unknown; it is supposed that has viral origin. First description of enation symptoms of grapevine originated from Buchenau (1891) in Germany. In Hungary it was first described by Lehoczky (1965). The symptoms are: delayed bud break, slow bushy growth of the shoots and presence of laminar or cup-shaped outgrowths (enations) on the underside of the eight to ten leaves at the base of the shoots. No vector is known; the disease spreads with propagation

material. The only identification method is the graft transmission to the indicator LN 33. Indexing, however, is highly unsatisfactory (Martelli, 1993).

Grapevine vein necrosis

In 1973 Legin and Vuittenez were discovered and described this virus-like disease (Martelli and Boudon-Padieu, 2006). In Hungary it was reported by Lehoczky *et al*. (1986b). Symptoms consist of necrosis of the vein lets on the underside of the leaf blade. The necrotic reactions develop first in the leaves at the base of the shoots and then, as the shoots grow, on the younger leaves. With time, necrotic spots also appear on the upper side of the leaf blade. Severe strains may induce necrosis of tendrils and dieback of green shoots. An almost complete cessation of growth ensues, and the indicator may die (Martelli, 1993; Martelli and Boudon-Padieu, 2006). The causal agent of vein necrosis is not known; the disease spreads by infected propagation material and by grafting. No herbaceous host plants are known. Grafting is done to *Vitis rupestris x Vitis berlandieri* 110 R (Martelli, 1993). Bouyahia *et al.* (2005) found, that GRSPaV may responsible for the appearance of vein necrosis symptoms on the indicator 110 R.

Grapevine vein mosaic

Vuittenez and co-workers observed of a type of mosaic of grapevines in 1966 which appears to be independent of grapevine fanleaf, but the pathogen was never characterized (Martelli and Boudon-Padieu, 2006). The disease was found also in Hungary (Lehoczky *et al*., 1984a). Infected plants show pale green mosaic along the main veins or the smaller ones, producing often a vein banding. In the most sensitive varieties, areas of the leaf blade may become necrotic, but these necroses not affect the veins as it is the case with vein necrosis. The disease may spread by grafting and vegetative propagation. *Vitis riparia* cv. Gloire de Montpellier is used as indicator variety (Martelli and Boudon-Padieu, 2006).

Overview and conclusion

In Hungary the grape and vine culture based on more hundred years traditions. Virus and virus like disease have been continuously threatened the yield and the quality, at least causing the early mortality of stocks. The high losses caused by viruses have led to intensified study of this group of pathogens and improve methods of control. Five decades ago Lehoczky and his colleagues played a leading role in organizing a certification scheme for the production of virus-free nursery source materials. They reported the occurrence of virus diseases mainly in Hungarian language, therefore they results has not get a large publicity. The virus eradication programme is still continuously run, but by the international exchange of propagating materials we have to calculate with the appearance of new pathogens. Continuing research is necessary to identify the new diseases. The demands of fruit-growing practice dictate that the future trend of research should be towards the diagnostic methods essential in producing virus-free propagating materials. The serological detection of grapevine viruses started some years ago in Hungary. From the studies it is concluded, that GFkV and GLRaV will play the major role in the degradation of grapevine stocks in Hungary. Among the 277 leaf samples 90 cases gave positive results, complex virus infection was detected in 14 samples.

The majority of GFkV infected twenty six samples originated from the North and Southwest part of Hungary. Thirty three samples proved to be infected by GLRaV-1, -2, -3. (Cseh *et al*., 2010; Cseh *et al*., 2011).

The serological testing of grapevine viruses sometimes could be difficult. A special extraction buffer should be used, because of inhibitors influencing the serological reactions. Now the introduction of molecular diagnostic methods seems to be essential for the correct identification even for the formerly characterized viruses. Because of the changes in classification are contiguous depend on the newest molecular results, it must follow this trends and start the molecular testing the grapevine viruses in Hungary too.

Using these techniques it would be possible to enhance the reliability of conventional diagnostic methods as well as to discover small differences among isolates.

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