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THE DEMONSTRATION OF THE GFLV *NEPOVIRUS* ISOLATES ON NATURALLY INFECTED GRAPEVINE CULTIVARS AND EVALUATION OF VARIABILITY WITHIN GENOME REGION ENCODING MOVEMENT PROTEIN

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Abstract

EICHMEIER, A., BARÁNEK, M., PIDRA, M.: *The demonstration of the GFLV Nepovirus isolates on naturally infected grapevine cultivars and evaluation of variability within genome region encoding movement protein.* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 3, pp. 35–44

In this work, the infection of *Grapevine fanleaf virus* (GFLV) disease was described on the base of symptomatic differences within eight grapevine plants of six grape ciltivars with positive tests on GFLV. Among them, cultivars Kodrjanka, Pamjati Negrula, Kišmiš Lučistyj were planted in wine region of the South Moravia (Czech Republic), three infected grapevine cultivars (URS, Cinsaut, Dimrit) included in this study originating from Italy. Except symptomatic evaluation, the differences between isolates were emphasized at the genetic level too, exactly in the frame of RNA2 genomic region coding movement protein. The variability of the tested isolates within the eight plants was in the range from 86.59 to 97.61% at the nucleotide level. The results confirmed very high degree of similarity between virus isolates of GFLV within studied RNA2 region. This fact was assessed by the phylogenetic analysis of obtained sequencing data too.

Nepovirus, GFLV, sequence, symptomatology

Grapevine fanleaf virus is the one from the oldest known viruses on the grapevine (*Vitis vinifera* L.) (Martelli, 1986). It is thought that GFLV has coexisted with grapes since their earliest cultivation and has spread with the vegetatively propagated crop. This viral disease is spread less than another *Nepovirus Arabis mosaic virus* (ArMV) in the Czech Republic (Komínek and Holleinová, 2003). ArMV is assigned as quarantine virus in the Czech Republic but GFLV not yet.

Grapevine fanleaf virus (GFLV, genus *Nepovirus*, family *Secoviridae*) induces significant yield reduction and lowering of the quality of grapevine fruit and must as well as vine degeneration. It causes malformation to the leaves, shoots and fruits, whereas some strains cause yellow discoloration of the leaves. Berry set on infected vines is reduced. Yield loss of up to 80% (Martelli and Savino, 1988) has been reported, in addition to lower quality and a reduction in vineyard longevity. Damage and malformation vary depending on the grapevine species (and variety) and virus isolate (Walter, 1998). The symptoms on the leaves are very different depending on grape cultivar and seasonal influences. It displays distortions of leaves, ringspots, line patterns, vein banding, yellowish mottling, and mosaic in different cultivars. Infected grapevines exhibit foliar symptoms early in the season that tend to fade during the summer and fall. Plant-to-plant spread of the virus in the vineyard occurs only by the ectoparasitic dagger nematode *Xiphinema index* Thorne & Allen (Esmenjaud *et al.*, 1993) and *Xiphinema italiae* Meyl (Cohn *et al.*, 1970). The virus is also transmitted efficiently by grafting and via the distribution of infected vegetative propagation materials.

Grapevine fanleaf virus is typical by his segmented genome. The genome is the main component of virus particle. Virus particles have angular outline about 30 nm in diameter, containing a single protein species of Mr 56 000. The genome consists of two positive-sense ssRNA molecules (RNA1, RNA2) that encapsidated separately. Both genomic RNAs are covalently linked to their 5' ends to small viral protein (VPg) and they are polyadenylated in their 3' ends. 3'-NCR of both RNAs are identical for many nepoviruses (Le Gall *et al*., 1995).

The first complete macromolecules of RNA1 and RNA2 were sequenced in case F13 strain (Ritzenthaler *et al*., 1991; Serghini *et al*., 1990). The final lenght was 7342 and 3774 nucleotides for RNA1 and RNA2.

RNA1 and RNA2 are monocistronic and each encodes a single polyprotein that is processed proteolytically into functional proteins required to complete the virus life cycle.

The RNA2-encoded P2 polyprotein contains (from the N- to C-terminus) the domains for the homing protein ($2A^{HP}$), the movement protein ($2B^{MB}$), and the coat protein ($2C^{CP}$) (Fig. 1) (Margis *et al*., 1993). The $2A^{HP}$ localizes in the replication site and has been implicated in RNA1-dependent replication of RNA2 (Gaire *et al*., 1999). The $2B^{MP}$ is a movement protein and is found in tubules observed in the plasmodesmata (Ritzenthaler *et al*., 1995). The $2C^{CP}$ is a multifunctional coat protein that is important in specific transmission by *X. index* Thorne & Allen, encapsidation of genomic RNAs, and systemic spread in plants (Andret-Link *et al*., 2004; Belin *et al*., 2001; Callaway *et al*., 2001; Hewitt *et al*., 1958).

GFLV was observed in various molecular variants in many countries of Europe, Africa, Middle East, North and South America (Bashir *et al*., 2007; Fattouch *et al*., 2005; Liebenberg *et al*., 2009; Mekuria *et al*., 2009; Naranghi-Arani *et al*., 2001; Pompe-Novak *et al*., 2007; Radaelli *et al*., 2009); Vigne *et al*., 2004. GFLV was described in the Czech Republic only by Komínek *et al*. (2006) that described the mild isolate HV5. The most of studies above is focused on special regions on the RNA2 molecule. The studies focused on characters of variability in the frame of genes localizated on RNA1 molecule are rather exemptions.

There is close relative of GFLV and that is ArMV. The symptoms of both nepoviruses are very similar and they depends on grape cultivar. The spatial spread of both viruses in the Czech Republic is not same. GFLV is not so numerous like ArMV (Komínek and Holleinová, 2003). Thereby ArMV is bigger threat than GFLV in the Czech Republic. It can be caused by vector of ArMV *X. diversicaudatum* that is natural in the Czech Republic but the vector of GFLV *X. index* Thorne & Allen wasn't discovered in the Czech Republic yet (Kumari *et al*., 2005). We suppose that GFLV spread in the Czech Republic is realized mainly by vegetative propagation. Another theoretical danger can be fact that the existence of observed recombinants between ArMV and GFLV (Mekuria *et al*., 2009) can be spread by nematodes that are natural in the Czech Republic.

Five infected grapevines of three grape cultivars (Kodrjanka, Pamjati Negrula, Kišmiš Lučistyj) planted in the South Moravia (Czech Republic) and three infected Italian grapevines of cultivars URS, Cinsaut and Dimrit were included to this study. There were included also obtained ArMV isolates because those shared close phylogenetic relationship with GFLV. The isolates S10 (ArMV) and K4 (ArMV) were the most important obtained ArMV isolates because their symptoms were absolutely indefinable from GFLV. Those isolates were obtained from grape cultivar Pinot Noir in another vineyard production in the wine region Moravia.

Presented paper contains the detail description of infected plants via its symptomatic manifestations on the individual grape cultivars.

These individual symptoms were subsequently linked to detail genetic analysis of genome region of RNA2 which coding $2B^{MP}$ movement protein at the nucleotide and amino acid level. The $2B^{MP}$ protein is the most homologous point at the RNA2 strand between GFLV and ArMV (Wetzel *et al*., 2002).

MATERIAL AND METHODS

Plant material

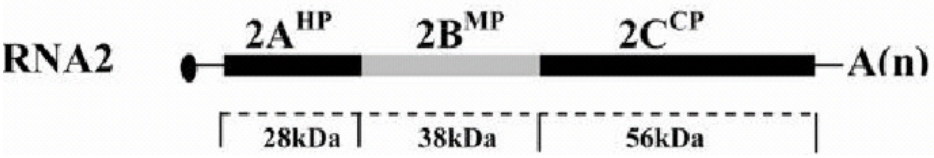
The isolates were collected since 2008 according to detecting tests by RT-PCR method. The finally studied natural isolates were sourced from grape cultivars Kodrjanka, Pamjati Negrula, Kišmiš Lučistyj. These cultivars has origin in Moldova but those are grown in the South Moravia (CZ) more than 20 years. There were added 3 provided isolates from Mediterranean Agronomic Institute of Bari (Italy) to this collection, URS, Cinsaut and Dimrit. The collection of isolates is recorded in Tab. I.

RT-PCR

Master mix for reverse transcription consists of 5 µl of the crude total nucleic acid extracts (TNAs) were primed with 1 µg of oligo (dT) after heat denaturation. Subsequently were reverse-transcribed with 200 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen Corporation, Groningen, The Netherlands) in 50 µl reaction for 1 h at 39 °C. The used PCR were published by Wetzel *et al*. (2002).

Sequencing of PCR products

PCR amplicons of expected lengths were cutted from the agarose gel and those were purified by NucleoSpin Extract II (Mascherey-Nagel). Nucleotide sequencing was done by BigDye® Terminator v3.1 (Applied Biosystems). The products of sequencing reaction were separated using genetic analyser ABI-PRISM 310 (Applied Biosystems). The sequencing was done in 5' and 3' direction for



1: *Diagramatic representation of genomic RNA2 of GFLV. The names of processed proteins are indicated above RNA and their estimated sizes are indicated below RNA. 2A^{HP}: homing protein, 2B^{MP}: movement protein and 2C^{CP}: coat protein. RNA2 has 5'- and 3'- noncoding regions flanking the polyprotein (represented by solid line), a VPg protein (closed circle) covalently linked to the 5'NCR and poly(A) tail (represented by A$_{[n]}$) at their 3' end*

I: *List of the RNA2 sequences of Grapevine fanleaf virus (GFLV), Arabis mosaic virus (ArMV) and Grapevine deformation virus (GDefV) used in this study*

Virus	Genome	Accession	Host	Cultivar	Isolate	Size (nt)	Country of origin	Reference
GFLV	RNA2	NC_003623	V. vinifera	Muscat	F13	3774	France	Serghini *et al.*, 1990
GFLV	RNA2		V. vinifera	Kodrjanka	KO1	290	Czech Republic	This study
GFLV	RNA2		V. vinifera	Kišmiš Lučistyj	KML51	290	Czech Republic	This study
GFLV	RNA2		V. vinifera	Pamjati Negrula	PN32	290	Czech Republic	This study
GFLV	RNA2		V. vinifera	Pamjati Negrula	PN33	290	Czech Republic	This study
GFLV	RNA2		V. vinifera	Pamjati Negrula	PN35	290	Czech Republic	This study
GFLV	RNA2		V. vinifera	URS	UR11	290	Italy	This study
GFLV	RNA2		V. vinifera	Cinsaut	55TK	290	Italy	This study
GFLV	RNA2		V. vinifera	Dimrit	63TK	290	Italy	This study
GFLV	RNA2	DQ386866	V. vinifera	...	HV5	814	Czech Republic	Komínek *at al.*, 2006
ArMV	RNA2	NC_006056	V. vinifera	Pinot Gris	NW	3820	Germany	Wetzel *et al.*, 2001
ArMV	RNA2		V. vinifera	Pinot Noir	S10	290	Czech Republic	This study
ArMV	RNA2		V. vinifera	Pinot Noir	K4	290	Czech Republic	This study
ArMV	RNA2		N. clevelandii	...	OL1	290	Czech Republic	This study
ArMV	RNA2		V. vinifera	…	AR/G2	290	Germany	This study
ArMV	RNA2		V. vinifera	…	AR/G4	290	Germany	This study
ArMV	RNA2		N. clevelandii	…	AR/S3	290	Switzerland	This study
GDefV	RNA2	AY291208	V. vinifera	Dimrit	N66	3753	Turkey	G.-Sabanadzovic *et al.*, 2005

each isolate. The amplification primers were used as Wetzel *et al.* (2002) described.

Phylogenetic analysis

Multiple sequence alignments and pairwise comparison of RNA2 (2B^{MP}) of GFLV, ArMV and GDefV (Table I.) were performed with CLC Main Workbench 5.0 (CLC bio) and nucleotide and amino acid sequence identity levels were calculated using the same software. The phylogenetic analysis was performed using the neighbor-joining (NJ) method that CLC Main Workbench 5.0 contains too. A bootstrap value for each node of NJ trees was calculated using 1000 bootstrap replicates and a consensus tree was displayed by this software.

RESULTS AND DISCUSSION

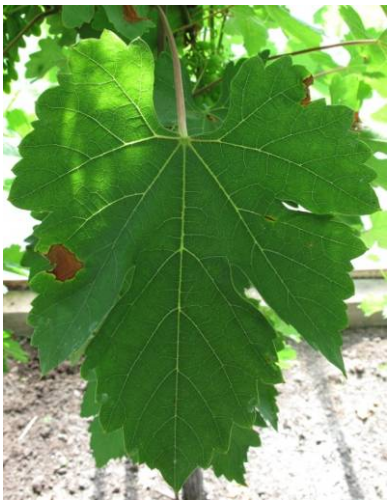
Symptomatology, the demonstration of studied isolates on specific grape cultivars

GFLV-infected grapevines often show a patchy distribution in diseased vineyards, as a result of a plant-to-plant virus transmission by the ectoparasitic dagger and its limited movement in the soil. GFLV causes a variety of symptoms in grapevines that differ in type and severity (Martelli and Savino, 1988). There is a clear confirmation that specific isolates has different symptoms on the grapevines. Some isolates are called "mild" isolates generally. They show no visual symptoms on plants at present. But it doesn't mean that these isolates won't show symptoms in the future. The testing of some plants infected by mild isolates can prove that the virus concentration is relatively high in plants.

We suppose that the mild isolates can change nature and then can be changed to plant destructive form. The aggressive form can generally cause the quick decay of all plant (proved at grape cultivar Pamjati Negrula and Kišmiš Lučistyj).

Isolate KO1

The isolate KO1 is the clear example of the mild isolate. It was obtained from the grape cultivar Kodrjanka planted more than twenty years in this location, Mendeleum, Faculty of Horticulture in Lednice. The isolate KO1 (Fig. 2) assigned only a slight yellow spots which are visual only under strong emission of light. The spots began distinct at the some leaves, it was linked with the late vegetation period (VI–VII months). Some leaves were lighter and there were dark green maps on these leaves. There were malformation of leaves very rarely and some changes in a numbers of lobes were presented. The infected plant did not show another visible changes caused by virus effect. This isolate had no other symptoms yet, the plant produced good yields of fruits every year. Total RNA was isolated from the grapevine at X/2008. The grapevine showed the symptoms less (2008) than in this year (2010).



2: *Symptoms of isolate KO1 on the grape cultivar Kodrjanka, there were slight deformation of leaf lobes and slight light green lesions*

Isolates PN32, PN33, PN35

The isolates from group of PN were obtained from grape cultivar Pamjati Negrula. Those were located very close at the same vineyard, thus the close phylogenetic relation can be supposed. The isolation of total RNA was done at X/2008, the grapevines with isolates PN33 and PN35 assigned poor health. Now, grape cultivar Pamjati Negrula with the isolate PN33 and PN35 are absolutely necrotized, only the rootstock is growing under point of grafting. In this case there is maybe very aggressive isolate or this grape cultivar is very sensitive to GFLV. The grapevine where the isolate PN32 was obtained was observed at VI/2010. Yellow spots and a lot of leaves malformations (Fig. 3) were more visible than in the case of KO1 isolate on the grape cultivar Kodrjanka.

3: *Symptoms of isolate PN32, strong leaf malformation, changes in the number of leaf lobes and light green mottling*

The isolate KML51 was recovered from the grape cultivar Kišmiš Lučistyj in the same vineyard like the isolates from PN group. The grapevine was cultivated in the same line (across 16 plants). Total RNA was isolated at IX/2009, the plant showed slight symptoms like chlorosis and yellow spots but generally health of this plant was possible to evaluate as a good. At the end of VI/2010 grapevine has strongly limited growth and strong deformation of leaves occurred (Fig. 6). Leaves were small with a strong chlorosis and slight yellow spots, deformations of leaves were distinctive in the case of culture grape cultivar (Fig. 4) and also in the case of growing up rootstock (Fig. 5). This observation implies that destructivity of GFLV can be influenced by weather character in individual years.

4: *Symptoms of isolate KML51 on the grape cultivar Kišmiš Lučistyj, leaf malformation and sharp yellow spots*

5: *Symptoms of isolate KLM51 on rootstock with grape cultivar Kišmiš Lučistyj*

6: *The grapevine of variety Kišmiš Lučistyj with rootstock, plant with reduced vitality*

Italian isolates UR11, 55TK, 63TK

These isolates were provided by Mediterranean Agronomic Institute of Bari (Italy). On the base of observed symptoms and results of genetic analysis it seems that isolates has mutually a different phylogenetic origin.

The isolate UR11 was obtained from grape cultivar URS. The leaves have a weak fanleaf shape (it can be specific for this grape cultivar, these symptoms were observed at all leaves) with light diffuse spots. But generally it's possible to state that the plant was very vigorous (Fig. 7). The detection by RT-PCR suggested high concentration of the viral RNA in tested tissues.

As showed results of RT-PCR tests, the isolate 55TK was present in the grape cultivar Cinsaut in high concentration. The symptoms were showed as slight deformations of leaves with light spots (Fig. 8). These were basically symptoms with mild negative effect on plants. This can be consequence of the age of plants which were planted from cuttings obtained at X/2008 from Italy. Thus, it's probably too early to determine the plant as vigorous or 55TK isolate as mild. As show our obtained experiences, forthcoming seasons will resolve it. Now it's not clear how will be these plants vigorous in forthcoming seasons yet.

The third isolate from Italy was signed as 63TK. It was recovered from grape cultivar Dimrit. From point of view of symtomatology this isolate wasn't showed so aggressive. The infected grapevine showed light green lesions on the leaves and there were visual irregular lobes (Fig. 9). Interestingly, we had a serious problem to detect the virus in 63TK isolate. The problem was maybe caused by the fact that the 63TK is not clear GFLV but close related *Nepovirus Grapevine deformation virus* (GDefV) which was firstly described by Cigsar *et al.* (2003). We recognised the same symptoms on the infected plant as Cigsar *et al.* (2003). All of three Italian isolates were preserved in host grape cultivars and showed satisfactory vitality for transformation to *in vitro* cultures too.

7: *Symptoms demonstrated on the grape cultivar UR11 with unusual shape of leaves and with light diffuse spots*

8: *The preview of isolate 55TK on the grape cultivar Cinsaut with slight malformations of leaves*

9: *The leaf of grape cultivar Dimrit infected by isolate 63TK, probably symptoms of GDefV (Cigsar et al., 2003)*

Phylogenetic assessment of genome region coding movement protein $2B^{MB}$

The five Czech GFLV isolates (KO1, PN32, PN33, PN35, KML51) and three obtained isolates from Italy (UR11, 55TK, 63TK) were sequences in the part of RNA2 coding movement protein. Movement protein is coded in the part 1004–2047 nts of RNA2 (Serghini *et al*., 1990), used primers amplify RNA2 region 1322–1609 nts. Thus expected amplicons were approximately 290 nucleotides in size.

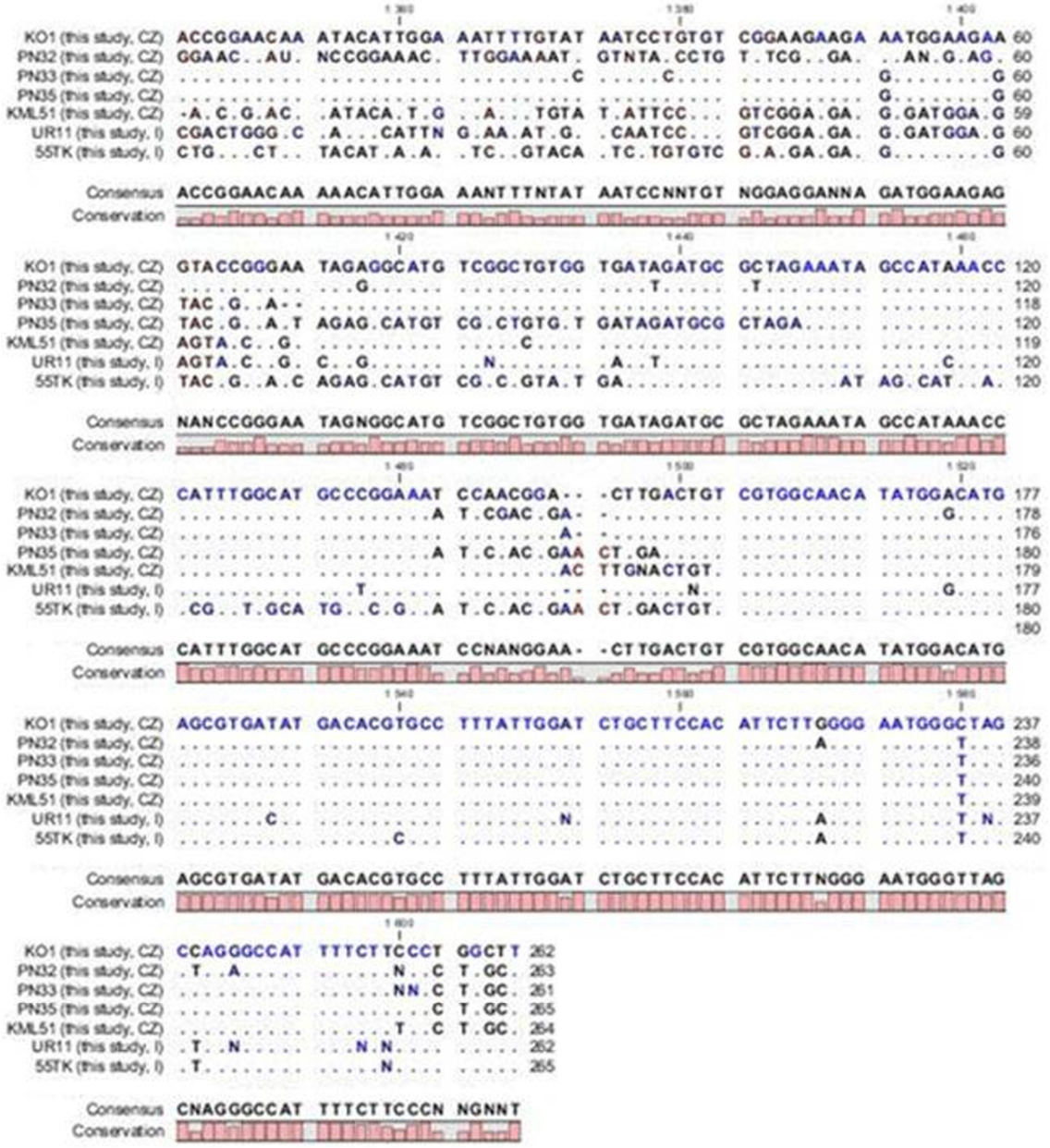
These sequences were analysed by software CLC Main Workbench 5.0 (CLC bio), multiple alignment method was done (Fig. 10). Sequences of GFLV isolates showed the homology in range from 86.59 to 97.61% by pairwise analysis (Fig. 11). The lowest homology within analysed genome portion was noticed in the case of isolate PN32 if compared with the other isolates. The number of gaps ranged from 0 to 18. The isolates obtained in the Czech Republic shared the homology in range from 85.16 to 97.61% including isolate HV5 (Komínek *et al*., 2006). The homology between sequences only in frame of the Italian isolates was in range from 84.52 to 97.61%. These differences assigned that the obtained Italian isolates had different phylogenetic origin. The variability at the amino acid level was in range from 87.21 to 100% of identity but only in the frame of studied GFLV isolates.

The homology of isolate PN32 with isolates KO1, PN33, UR11 and 55TK reached value 87.21%. The isolates KO1, UR11 and 55TK reached 100% similarity at the aminoacid level.

We suppose that the isolate 63TK should be the same like the isolate N66 (Cigsar *et al*., 2003). The result of sequencing of 63TK wasn't relevant because used primers amplified the part of genomic mRNA of grapevine and the part of RNA2 of *Nepovirus*. This is a reason why 63TK isolate wasn't included in the phylogenetic analysis. The amplicon of *Nepovirus* was sequenced in 5'–3' direction by primer M2, then the homology of 63TK nucleotide sequence with another sequences in NCBI database was possible. The most similar isolate from the NCBI database was determined Gen. Bank. Acc. Nos. FJ544925 which was obtained in France from grape cultivar Gewurztraminer (Vigne *et al*., 2009). The amplification reactions of 63TK isolate were unsuccessful from other genomic regions GFLV and ArMV from RNA2 (from coat protein $2C^{CP}$) and GFLV (from RNA1 helicase $1B^{Hel}$ and RNA-dependent RNA polymerase $1E^{Pol}$). As mentioned above, genome region coding movement protein in the frame of RNA2 was described the highest homology among GFLV and ArMV (Wetzel *et al*., 2001). It was probably the reason why the primers M2/M3 were used in this study gave amlicon in case of 63TK isolate. The primer couple M2/M3 is very suitable for detection of each from these three nepoviruses. For the future research it could be interesting to study the occurrence of GDefV in the Czech Republic.

The enlargement of collection of *Nepovirus* isolates (Tab. I) provided results of variability studied genomic region in range from 71.65 to 97.61%. Enlargement consist mainly of ArMV isolates, which were added because of known phylogenetic relation with GFLV. Added ArMV isolates originating from various countries of Middle and Western Europe but were sequenced in our laboratory. From point of view of results, the lowest percent identity assigned isolate N66 of GDefV (Cigsar *et al*., 2003) compared with the GFLV isolates. Generated dendrogram (Fig. 12) clearly showed the separated clusters of ArMV and GFLV isolates. It's an interesting that Turkish isolate GDefV N66 was clustered with GFLV mild isolate HV5 (Komínek *et al*., 2006) at the middle point among GFLV and ArMV.

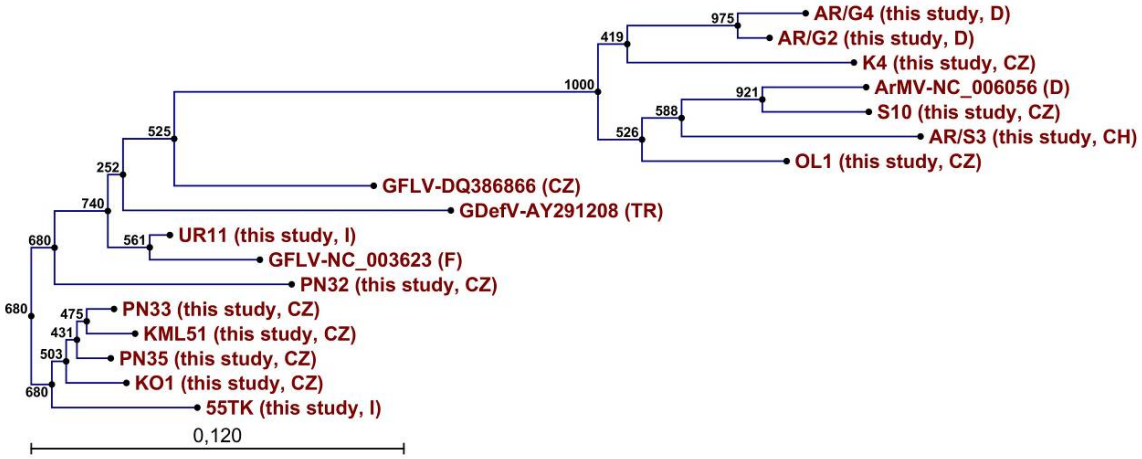
With respect to very close phylogenetic relation of nepoviruses GFLV, ArMV and GDefV and their possible recombination (Vigne *et al*., 2009; Mekuria *et al*., 2009) is logical to suppose that these viruses has same phylogenetic origin. This supposition confirms the fact that the symptoms of specific isolates of viruses (phylogenetically close or not) are practically unrecognizable apart. No one from studied GFLV isolates did assigned classical symptoms like fanleaf that is even reflected in the name of GFLV. Within the group of analysed grapevine isolates in this work we even recognised this symptom as a typical for ArMV. This symptom was observed on the grape cultivar Pinot Noir where the isolates S10 (South Moravia/CZ) (Fig. 13) and K4 (South Moravia/CZ) were obtained.



10: *The instance of the multiple alignment analysis of obtained GFLV isolates, there are MP-derived sequences in genome portion approximately 1322–1609 nucleotides (RNA2). The dots means identical nucleotides and the lower line is the consensus with the degree of conservation.*

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
GFLV-NC_003623 (F)	1		86,23	90,24	95,92	89,88	89,24	90,28	89,60	87,89	88,84	76,59	75,20	74,02	72,94	74,90	77,20	76,77
GDefV-AY291208 (TR)	2	2		82,59	87,04	85,14	84,92	86,69	85,26	80,54	84,19	75,00	74,41	71,65	72,94	72,91	73,71	75,98
GFLV-DQ386866 (CZ)	3	1	1		91,06	86,29	86,06	87,85	86,40	85,16	84,52	77,78	75,98	75,98	74,90	77,29	77,69	78,74
UR11 (this study, I)	4	0	2	1		93,12	92,43	93,93	92,00	89,45	92,43	78,97	77,56	76,77	76,08	77,69	78,40	78,35
KO1 (this study, CZ)	5	2	4	3	2		96,83	96,79	95,63	90,27	92,86	77,17	77,17	76,47	73,44	74,31	74,21	76,08
PN35 (this study, CZ)	6	6	6	5	6	6		97,61	97,23	90,35	93,70	77,25	76,56	76,56	74,22	74,41	73,62	76,17
PN33 (this study, CZ)	7	2	2	1	2	4	4		97,60	89,49	93,65	78,17	76,77	76,38	74,12	74,90	74,50	76,77
KML51 (this study, CZ)	8	5	5	4	5	7	5	3		88,85	93,31	78,35	76,26	76,56	73,54	74,02	74,02	77,25
PN32 (this study, CZ)	9	15	15	14	15	15	15	15	18		86,59	75,48	74,81	73,66	71,86	73,08	72,69	75,19
55TK (this study, I)	10	6	8	7	6	6	6	6	7	19		76,86	76,17	76,08	75,39	74,12	74,02	76,56
AR/G2 (this study, D)	11	9	7	8	9	11	9	7	8	20	9		96,85	88,14	87,75	88,00	88,00	90,08
AR/G4 (this study, D)	12	12	10	11	12	10	10	10	13	21	10	7		88,89	87,06	88,05	88,05	89,33
K4 (this study, CZ)	13	16	14	15	16	16	14	14	15	25	12	9	6		81,50	83,27	84,86	85,71
AR/S3 (this study, CH)	14	13	11	12	13	13	9	11	12	22	9	4	7	9		88,49	86,51	86,56
S10 (this study, CZ)	15	10	8	9	10	12	10	8	11	21	12	3	4	8	5		93,52	90,80
ArMV-NC_006056 (D)	16	9	9	10	9	11	11	9	12	22	11	4	5	9	6	1		89,20
OL1 (this study, CZ)	17	13	11	12	13	13	11	11	10	22	11	4	5	7	4	3	4	

11: *Pairwise sequence comparisons were done using the Maximum Matching program in CLC Main Workbench 5.0 (CLC bio). The results are shown in percent of homology and the numbers of gaps between obtained GFLV and ArMV sequences and ref. sequences of GFLV, ArMV and GDefV were added.*



12: *Dendrogram depicting phylogenetic relationships among the obtained GFLV and ArMV isolates compared together and to ref. sequences of GFLV, ArMV and GDefV (Tab. I). Neighbor-joining analysis with the bootstrapping of 1000 replicates was performed. The scale bar shows the number of substitutions per nucleotide.*

13: *Symptoms of ArMV isolate S10 recovered from grape cultivar Pinot Noir, it showed very strong fanleaf degeneration. The grapevine was located in the South Moravia.*

Published results can be important mainly for grapevine growers because of emphasized symptoms each three nepoviruses. Generally, all the aspects mentioned above are still fertile areas of research which will be further explored and develop within future scientific program of Mendeleum – Institute of Genetics and Plant Breeding in Lednice.

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