Acta virologica **57**: 353 – 356, 2013 doi:10.4149/av_2013_03_353

Analysis of a short genomic region of Grapevine leafroll-associated virus 1 (GLRaV-1) reveals the presence of two different molecular groups of isolates in Slovakia

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Received April 29, 2013; accepted July 3, 2013

Summary. – Although grapevine leafroll-associated virus 1 (GLRaV-1) is one of the most important agents of the grapevine leafroll disease, the data about its molecular variability are scarce. In order to assess the GLRaV-1 diversity in Slovakia, the sequence of a genome region encoding the central part of the capsid protein (CP) gene was determined from 18 GLRaV-1 isolates. Despite the fact that the analysis targeted a relatively short portion of the genome, comparison of obtained sequences has revealed the nucleotide identities between Slovak isolates ranging from 83.0–100%. Phylogenetic analysis indicated the presence of two distinct molecular groups of GLRaV-1 in Slovakia.

Keywords: leafroll; genetic diversity; RT-PCR; phylogenetic analysis

GLRaV-1 (the genus *Ampelovirus*, the family *Closteroviridae*) is one of the most important and widespread agents associated with the leafroll disease of grapevines (Habili *et al*., 2007; Martelli *et al*., 2012). Based on the only known complete genome sequence (Rott and Belton, unpublished, GenBank Acc. No. NC_016509), the GLRaV-1 genome is ca. 18.6 kb in size and contains ten open reading frames encoding distinct proteins (Fazeli and Rezaian, 2000). GLRaV-1 is transmitted by grafting and by mealybugs or scale insects (Martelli *et al*., 2012; Sforza *et al*., 2003; Habili *et al*., 2007).

Characteristic symptoms include the inward rolling of leaves in late summer (Rayapati *et al*., 2008). Depending on the virus isolate, grapevine cultivar and environmental conditions, leafroll virus infections can negatively influence the yield, sugar content and acidity of the berries, physiological performance of plants, their resistance to biotic and abiotic stress as well as length of growing cycle and the vigor (Guidoni *et al*., 1997; Moutinho-Pereira *et al*., 2012).

Genetic variability is an essential feature of RNA viruses. An understanding of the diversity of viral populations has a significant role in the epidemiology of the virus and constitutes an important prerequisite to develop an accurate diagnosis and efficient control strategies (Moury *et al*., 2006). Although GLRaV-1 is a common viral pathogen of grapevines worldwide, only few studies of GLRaV-1 molecular variability have been reported (Little *et al*., 2001; Komínek *et al*., 2005; Alabi *et al*., 2011).

In this work a set of Slovak GLRaV-1 isolates was studied through the partial sequence analysis, assessing, for the first time, the genetic diversity of this pathogen in Slovakia.

Materials and Methods

GLRaV-1 isolates were obtained from randomly sampled grapevine plants (*Vitis vinifera* L.) in different vineyards across Slovakia in 2011–2012. The isolates were recovered from different

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Abbreviations: GLRaV-1 = grapevine leafroll-associated virus 1; CP = capsid protein

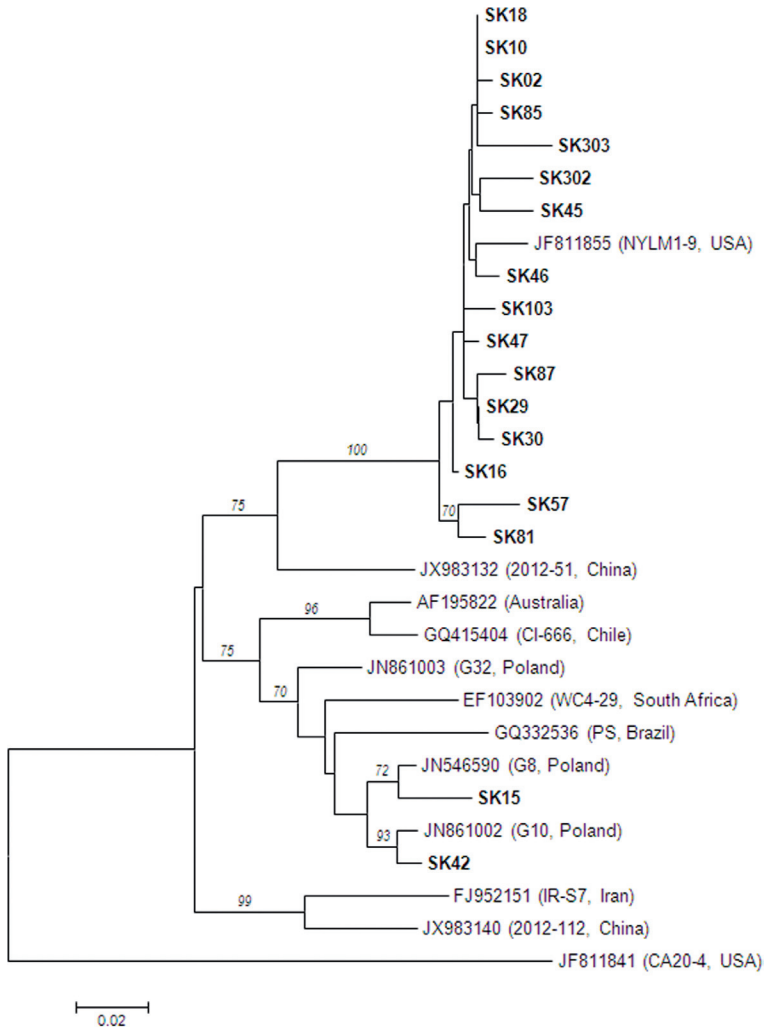

Fig. 1

Phylogenetic tree of GLRaV-1, generated from the 241 nucleotides long CP sequences of the Slovak isolates determined in this study (bold) and selected GLRaV-1 sequences retrieved from the GenBank database, http://www.ncbi.nlm.nih.gov (Acc. No. is followed by the isolate name and country of origin)

The scale bar indicates a genetic distance of 0.02. Bootstrap values >70% (1,000 bootstrap resamplings) are indicated on the branches. The tree was constructed by the neighbor-joining method, using the p-distance model.

independent vineyards and cultivars (Table 1). The preliminary screening of the GLRaV-1 presence in the grapevine plants was performed by DAS-ELISA, using commercial antibodies (Bioreba, Switzerland).

For molecular analysis, total RNAs were extracted from cortical scrapings from dormant canes using a NucleoSpin RNA Plant kit (Macherey-Nagel, Germany) following the manufacturer's instructions. A two-step RT-PCR protocol was used. The first-strand cDNA was synthesized by reverse transcription of total RNAs using random hexamer primers (Promega, USA) and the PrimeScript (MMLV) reverse transcriptase (TaKaRa, Japan) used as recommended by the supplier. A GLRaV-1-specific primer pair LR1f (5′-TGAAGGGRCCGGGAGGTTAT-3′, sense) and LR1rev (5′-TTACCCATCACTTCAGCAC-3′, antisense) (E. Angelini, unpublished results) were used to amplify ca. 280 bp fragment from the central part of the capsid protein (CP) gene. PCR was performed using the EmeraldAmp MAX PCR Master Mix (Takara, Japan) under the following conditions: denaturation 98°C 1 min, 35 cycles of 98°C 10 sec, 55°C 30 sec, and 72°C 30 sec and final elongation 72°C 5 min. RT-PCR products were purified using the Wizard Preps DNA purification system (Promega, USA) and directly sequenced by priming the sequencing reaction with the same oligonucleotides as used for PCR. After the primer sequences were removed, we have

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          84                                                                163
SK#       SLRPSRPSTGQRSGDSEVARREMGDKLKRTFNIAEIFTNPEMNIIFEPPKDMEVSVVVPTGPGLVTPAVATAISTELKNL
SK30      .........S.......................................................................
SK57      ..........................................................................A.....
SK81      ..............................S.....................................................
SK302     ..................I.....................................................................
SK303     .IP.....................................................................P.........
SK15      T..L........Q.............................V.....................................
SK42      T..L.....................................V.....................................

(# = SK2, SK10, SK16, SK18, SK29, SK45, SK46, SK47, SK85, SK87, SK103)
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Fig. 2

Multiple alignment of the amino acid sequences of the central part of the capsid protein of Slovak GLRaV-1 (amino acid positions 84–163 of the CP)
Identical amino acids are indicated by dots.

used a portion with a length of 241 nucleotides (nt) for further analyses (nt positions 13,295–13,535 based on the complete sequence NC_016509). Sequence analyses were performed using Molecular Evolutionary Genetics Analysis [MEGA v. 5.1; (Tamura *et al*., 2011)]. The nucleotide sequences reported herein have been deposited in the GenBank database under Acc. Nos. KC867254-KC867271.

Results and Discussion

The survey of GLRaV-1 occurrence in Slovakia using the commercial antibodies in DAS-ELISA has indicated its frequent occurrence in grapevines (M. Glasa, unpublished data). Eighteen grapevine samples representing different vineyards in 7 Slovak localities were tested positive in RT-PCR using the GLRaV-1-specific primer set, generating the product of ca. 280 bp from the central portion of the CP gene. To assess the genetic variability of GLRaV-1, the sequences of 18 isolates were determined (Table 1). Based on multiple alignment, all obtained sequences were found to be fully collinear and without indels. Despite the analysis of a relatively short genome portion, the nucleotide identities between Slovak GLRaV-1 isolates ranged from 83.0 to 100%. The overall divergence, including all of the analyzed Slovak GLRaV-1 isolates, was 0.049 (±0.006). The identities at the amino acid level reached 92.5–100%.

Phylogenetic analysis showed that the GLRaV-1 isolates divided into 2 molecularly distinct groups. The majority of the isolates (16 of 18) were comprised in the cluster together with isolates from USA (Fig. 1 and data not shown). This cluster can be identical to the Group 3 previously reported by Alabi *et al*. (2011). At the amino acid level, 12 of 16 Slovak isolates were identical (Fig. 2), although originating from different localities or genotypes. Two additional isolates (SK15 and SK42) were phylogenetically related to Polish, Brazilian and South African isolates (Fig. 1), previously classified as forming the Group 1 (Alabi *et al*., 2011). These 2 isolates differed from the other Slovak GLRaV-1 isolates by 3 specific amino acid substitutions ($Thr_{84}Ser$, $Leu_{87}Pro$, $Val_{127}Ile$, numbered according to the capsid protein of the NC_016509 isolate).

Table 1. List of the Slovak GLRaV-1 isolates characterized in this study

Isolate	Locality	Cultivar	GenBank Acc. No.
SK02	Čachtice	Pinot Gris	KC867271
SK10	Čachtice	Traminer	KC867270
SK15	Pezinok	Veltliner	KC867259
SK16	Pezinok	Muller-Thurgau	KC867263
SK18	Pezinok	Muller-Thurgau	KC867268
SK29	Pezinok	Saint Laurent	KC867256
SK30	Pezinok	Veltliner	KC867257
SK42	Pezinok	Chasselas	KC867260
SK45	Pezinok	Chasselas	KC867266
SK46	Pezinok	unknown	KC867264
SK47	Bratislava	Fruhroter Veltliner	KC867255
SK57	Okoč	unknown	KC867261
SK81	Topolčianky	Saint Laurent	KC867262
SK85	Topolčianky	Panonia Kincse	KC867269
SK87	Topolčianky	Chasselas Rose	KC867258
SK103	Svätý Jur	unknown	KC867254
SK302	Limbach	Veltliner	KC867265
SK303	Limbach	unknow	KC867267

A better knowledge of the virus genetic diversity and the characterization and tracing of virus populations provides a major contribution to understand the complexity and epidemiology of the pathogen (Moury *et al*., 2006). Detailed genetic analysis of virus isolates occurring in an area was shown useful to understand the geographic dynamics and genetic evolution of viral population (Sharma *et al*., 2011). Previously, only one Slovak GLRaV-1 isolate was characterized by partial sequencing of the HSP70 gene (Komínek *et al*., 2005).

In our work, by targeting a relatively short genomic region, spanning the central part of the CP gene, we were able to confirm a high molecular diversity within GLRaV-1 isolates in Slovakia. Based on obtained results, this genomic portion was proven to be sufficiently informative to distinguish different virus clusters (groups). However, as the conflicting phylogenetic signals depending on the analyzed genomic region have been reported in case of GLRaV-1 (Alabi *et al.*, 2011), the unambiguous intra-species classification would require the access to the full-length genome sequences.

Acknowledgements. The authors thank Dr. Elisa Angelini (CRA – VIT Centre for Research in Viticulture, Conegliano, Italy) for providing the primer sequences prior the publication. This work was supported by grant VEGA 2/0060/13 from the Scientific Grant Agency of the Ministry of Education and Slovak Academy of Sciences.

References

Alabi OJ, Al Rwahnih M, Karthikeyan G, Poojari S, Fuchs M, Rowhani A, Naidu RA (2011): Grapevine leafroll-associated virus 1 occurs as genetically diverse populations. Phytopathology 101, 1446–1456. http://dx.doi.org/10.1094/PHYTO-04-11-0114

Fazeli CF, Rezaian MA (2000): Nucleotide sequence and organization of ten open reading frames in the genome of Grapevine leafroll-associated virus 1 and identification of three subgenomic RNAs. J. Gen. Virol. 81, 605–615.

Guidoni S, Mannini F, Ferrandino A, Argamante N, Di Stefano R (1997): The effect of grapevine leafroll and rugose wood sanitation on agronomic performance and berry and leaf phenolic content of a Nebbiolo clone (Vitis vinifera L.). Am. J. Enol. Vitic. 48, 438–442.

Habili N, Komínek P, Little A (2007): Grapevine leafroll-associated virus 1 as a common grapevine pathogen. Plant Viruses 1, 63–68.

Komínek P, Glasa M, Bryxiová M (2005): Analysis of the molecular variability of Grapevine leafroll-associated virus 1 reveals the presence of two distinct virus groups and their mixed occurrence in grapevines. Virus Genes 31, 247–255. http://dx.doi.org/10.1007/s11262-005-3236-1

Little A, Fazeli CF, Rezaian MA (2001): Hypervariable genes in Grapevine leafroll associated virus 1. Virus Res. 80, 109–116. http://dx.doi.org/10.1016/S0168-1702(01)00343-4

Martelli GP, Agranovsky AA, Al Rwahnih M, Dolja VV, Dovas CI, Fuchs M, Gugerli P, Hu JS, Jelkmann W, Katis NI, Maliogka VI, Melzer MJ, Menzel W, Minafra A, Rott ME, Rowhani A., Sabanadzovic S, Saldarelli P (2012): Taxonomic revision of the family Closteroviridae with special reference to the grapevine leafroll-associated members of the genus Ampelovirus and the putative species unassigned to the family. J. Plant Pathol. 94, 7–19.

Moury B, Desbiez C, Jacquemond M, Lecoq H (2006): Genetic diversity of plant virus populations: towards hypothesis testing in molecular epidemiology. Adv. Virus Res. 67, 49–87. http://dx.doi.org/10.1016/S0065-3527(06)67002-4

Moutinho-Pereira J, Correia CM, Goncalves B, Bacelar EA, Coutinho JF, Ferreira HF, Lousada JL, Cortez MI (2012): Impacts of leafroll-associated viruses (GLRaV-1 and -3) on the physiology of the Portuguese grapevine cultivar, Touriga Nacional′ growing under field conditions. Ann. Appl. Biol. 160, 237–249. http://dx.doi.org/10.1111/j.1744-7348.2012.00536.x

Rayapati AN, O′Neil S, Walsh D (2008): Grapevine leafroll disease. In: WSU Extension Bulletin. [http://cru.cahe.wsu.edu/CEPublications/eb2027e/eb2027e.pdf].

Sforza R, Boudon-Padieu E, Greif C (2003): New mealybug species vectoring Grapevine leafroll-associated viruses-1 and -3 (GLRaV-1 and -3). Eur. J. Plant Pathol. 109, 975–981. http://dx.doi.org/10.1023/B:EJPP.0000003750.34458.71

Sharma AM, Wang J, Duffy S, Zhang S, Wong MK, Rashed A, Cooper ML, Daane KM, Almeida RP (2011): Occurrence of grapevine leafroll-associated virus complex in Napa Valley. PLoS One, 6: e26227. http://dx.doi.org/10.1371/journal.pone.0026227

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011): MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739. http://dx.doi.org/10.1093/molbev/msr121