Raspberry bushy dwarf virus in Slovenia - geographic distribution, genetic diversity and population structure

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Abstract Raspberry bushy dwarf virus (RBDV) is a long-known virus naturally infecting Rubus and grapevine. It is also one of the economically most important viruses of raspberries, but there are only a limited number of sequences covering a substantial part of the genome available in the databases. The aim of this study was: i) to study the geographic distribution of RBDV in Slovenia, and ii) to sequence RNA2 of several red raspberry and grapevine RBDV isolates and study their phylogeny and population structure. Geographic distribution studies were performed over a period of 13 years in three wine-growing regions of Slovenia (Primorska, Podravje and Posavje). The highest incidence of RBDV was found in Podravje (58.8%) and the lowest in Primorska (5.1%). Big differences were observed between Vipavska dolina (10.2%) and three other wine-growing districts of Primorska region (0.4–1.2%). Almost complete RNA2 sequences were obtained for four red raspberry isolates and seven grapevine isolates. Additionally, only coat protein sequences were obtained for three red raspberry isolates. Phylogenetic and population diversity analyses were performed on all available RBDV sequences. Phylogenetic analysis has shown clear differences in sequences from Rubus and grapevine that form two highly supported clades. In RNA2 analysis additional two sub-clades were found in grapevine clade. Two major subclades were identified also in the Rubus clade with further differentiation within these subclades. Purifying or stabilizing selection was found to be acting on both, CP and MP genes while few codons were found to be under positive selection.

Keywords RBDV · Population structure · Grapevine · Rubus · Genetic diversity · Selection pressure

Raspberry bushy dwarf virus (RBDV) is a well-studied virus found naturally in raspberries, blackberries and grapevines (reviewed in Martin et al. 2013). In red raspberries, RBDV has long been implicated in crumbly fruit disease, which has been shown to be more severe in mixed infections with one or more aphid transmitted viruses (Martin et al. 2013; Quito-Avila et al. 2014). RBDV, a sole member of the genus Idaeovirus, is pollen transmitted. Natural infection with RBDV has long been confined to Rubus spp. while experimental host range is wider (Jones et al. 1982). The RBDV presence in Rubus spp. has been reported from Europe, North America, South America, South Africa, Japan and New Zealand (Barbara et al. 2001; Dal Zotto et al. 2017; Dulić-Marković and Ranković 1992; Isogai et al. 2012; Kooyman et al. 1982; Matus et al. 2008; Mavrič et al. 2003; Quito-Avila et al. 2013; Spak and Kubelkova 2000; Strik and Martin 2003; Valasevich et al. 2011; Wall and Shamoun 1990; Wood 1995). It was found in different wild and cultivated Rubus plants (red and black raspberry, blackberry, blackberry-
raspberry hybrids, wild *R. idaeus* var. *strigosus*, *R. occidentalis*, *R. parviflorus*, *R. leucodermis*, *R. multibracteatus*, *R. glaucus*, *R. arcticus*) (Chambelain et al. 2003; Kokko et al. 1996; Martin et al. 2013; Quito-Avila et al. 2013; Wall and Shamoun 1990). In 2003 the first natural RBDV infection of non-*Rubus* host was reported for grapevine from Slovenia (Mavrič et al. 2003) and later also from Serbia and Hungary (Jevremović and Paunović 2011; Mavrič Pleško et al. 2012; Czotter et al. 2018). Additionally, a virus showing high sequence similarity to RBDV was found in citrus (Jevremović et al. 2003) and in black currant (James and Phelan 2017; Thekke-Veetil et al. 2017).

The genome of RBDV is bipartite single-stranded RNA encapsidated in quasi-isometric particles. RNA1 contains one large open reading frame (ORF) encoding a 188 kDa protein with motifs of viral RNA helicases and polymerases (Ziegler et al. 1992). RNA2 has two ORFs, the one at the 5′-end encodes a 39 kDa movement protein (MP) and the one at the 3′-end a 30 kDa coat protein (CP) (Natsuaki et al. 1991). Parts of the genome were sequenced for RBDV isolates originating from different countries but there is still a very limited number of sequences available in the database.

Studies of RBDV in Slovenia started in 2002 when the virus was first detected in red raspberries from collection plantation of Agricultural Institute of Slovenia. In 2003, the RBDV infection was confirmed in grapevine grafts of cvs. Laški Rizling (Italian Riesling) and Štajerska Belina by DAS-ELISA and immunocapture RT-PCR and a few grapevine and red raspberry isolates were further characterized (Mavrič et al. 2003; Mavrič et al. 2004; Mavrič Pleško et al. 2009). RNA2 sequences used for molecular characterization of these isolates are some of the few RBDV RNA2 sequences available.

In this study the presence of RBDV was surveyed in grapevine in three wine-growing regions of Slovenia during the period of 13 years. Selected grapevine and red raspberry samples were used for molecular characterization and phylogenetic analysis of RBDV. Additionally, the population structure of RBDV was studied using available CP, MP and RNA2 sequences.

Over a period of 13 years (2003–2015), 2505 grapevine samples were collected from vineyards in all three wine-growing regions of Slovenia (Primorska, Podravje, Posavje). For geographical distribution studies, 1393 samples (55.6%) originated from Primorska, 730 (29.15%) from Podravje and 382 (15.25%) from Posavje wine-growing regions. All samples were tested for RBDV by DAS-ELISA using Bioreba reagents according to manufacturer’s instructions. The highest incidence of RBDV was determined in Podravje region (58.8%) and the lowest in Primorska region (5.1%) The results are presented in Table 1. In Primorska region where 4 wine-growing districts are defined, considerable differences in the RBDV incidence were observed between Vipavska dolina (10.2%) and other wine-growing districts of this region (0.4–1.2%) (Table 1, Fig. 1).

Over 30 grapevine cultivars collected in all wine-growing regions were analyzed. The largest number of samples was collected in Italian Riesling, Refošk, Merlot, Malvazija, Chardonnay and Pinot Gris. The highest infection rate was detected in Chardonnay, Rizvanec, Šipon, and Zweigelt (80% or higher) followed by Rhein Riesling (72.3%) and Italian Riesling (53.5%). Most of the RBDV positive samples from Primorska were collected in Vipavska dolina (Table 1) where the infection was confirmed in Italian Riesling (59%), Sauvignon (38.7%) and Malvazija (2.6%). In Posavje, the infected cultivars were Italian Riesling and Rhein Riesling (27.1% and 59.1% respectively) while in Podravje the majority of tested cultivars were found to be infected. The widespread infections with RBDV in Podravje indicate that the infection in Slovenia might originate in this region and had spread to other regions through infected planting material of cultivars grown in all regions. This would explain the high infection rate in

| Wine-growing region/district | positive | negative | all | (%)|
|-----------------------------|----------|----------|-----|----|
| Primorska                   | 71       | 1322     | 1393| 5.1 |
| Vipavska dolina             | 64       | 566      | 630 | 10.2 |
| Kras                        | 1        | 252      | 253 | 0.4 |
| Goriška Brda               | 2        | 187      | 189 | 1.1 |
| Slovenska Istra            | 4        | 317      | 321 | 1.2 |
| Podravje                   | 429      | 301      | 730 | 58.8 |
| Štajerska Slovenija        | 429      | 301      | 730 | 58.8 |
| Posavje                   | 51       | 331      | 382 | 13.35 |
| Dolenjska                 | 33       | 140      | 173 | 19.1 |
| Bela Krajina            | 18       | 191      | 209 | 8.6 |

Table 1 Results of serological detection of RBDV in grapevine samples from different wine-growing regions of Slovenia
Vipavska dolina in comparison with the rest of Primorska since Italian Riesling is a predominant cultivar of Slovenia but in the Primorska region it is almost exclusively grown in Vipavska dolina.

In spite of importance of RBDV in raspberry production, there are only eight whole genome sequences and an additional seven sequences of complete or almost complete RNA2 available in the databases. Obtaining additional RNA2 sequences of RBDV from grapevine and red raspberry was the main aim of this study that allowed for better understanding of its diversity. Several grapevine and red raspberry samples, confirmed to be infected with RBDV by DAS-ELISA, were selected for diversity study. The selected grapevine samples were from different wine-growing regions while raspberry samples all originated from collection plantation of Agricultural Institute of Slovenia at Brdo pri Lukovici in central Slovenia. For the purpose of this study, all samples used for diversity study are referred to as isolates. The IC RT-PCR or total RNA extraction and RT-PCR were used to confirm RBDV infection of seven grapevine and seven red raspberry samples and to amplify two PCR products covering almost complete RNA2 of RBDV. The IC RT-PCR, RT-PCR, cloning and sequencing were performed as previously described (Mavrič Pleško et al. 2009). Sequences were analysed using Geneious version 8.1.8 and 11.1.4 (http://www.geneious.com, Kearse et al. 2012) and assembled into contigs. Almost complete RNA2 sequences (including complete ORFs for CP and MP) were obtained for seven grapevine and four red raspberry isolates and only CP sequences were obtained for three red raspberry isolates. Sequences were deposited in the GenBank under Accession Numbers KY417868 KY417881.

Phylogenetic relationships between the isolates were inferred from UPGMA clustering method. Trees were constructed from (1) near complete RNA2 alignment of 27 sequences with 2124 nucleotides (Fig. 2), and (2) CP gene alignment from 41 RBDV sequences with 822 nucleotides (Fig. 3); 14 obtained from this study and 27 from Genbank database (Table 2). Data were bootstrapped with 1000 re-samplings to test the robustness of the lineages in the trees. Analyses were performed using Tamura 3-parameter method implemented in MEGAX software (Kumar et al. 2018). Four sequences were used as an outgroup: DQ120126 (RBDV
RNA2 sequence from *Rubus multibracteatus*, China, Chamberlain et al. 2003), DQ100358 (CP gene of *Citrus idaeovirus*, USA, Derrick et al. 2006), KY399999 (RNA2 sequence from blackcurrant leaf chlorosis associated virus, USA, Thekke-Veetil et al. 2017), and KX838924 (RNA2 from blackcurrant leaf chlorosis associated virus, Canada, James and Phelan 2017). Sequences from raspberry and grapevine formed two distinct and highly supported clades (> 92%) in RNA2-based and CP-based phylogenetic trees (Figs. 2 and 3). All grapevine sequences clustered together in one clade. Lower isolate diversity was observed within grapevine clade of CP gene. Overall, no correlation between white and red cultivar isolates was noticed. The clade of RNA2 sequences from raspberry showed higher internal clustering with two major sub-clades, one with Slovenian and Ecuador sequences (93%), and the other with Belarus and UK sequences (100%). Further differentiation was observed also within these sub-clades. Higher diversity was also observed in phylogenetic tree of raspberry CP gene.

It has already been demonstrated that grapevine and raspberry RBDV sequences differ genetically and phylogenetically from each other. The distinction was first observed after the discovery of RBDV in grapevine (Mavric Plesko et al. 2009) and also by Valasevich et al. (2011) when RBDV raspberry sequences from Belarus and Sweden were compared to all available RBDV sequences. Phylogenetic analysis of RNA2 sequences performed in the study of Valasevich et al. (2011) confirmed clear differentiation between grapevine and raspberry sequences. Further differentiation was observed within raspberry clade. Analysis performed in our study confirmed this differentiation with additional sequences in each of the two clades. No obvious geographical differentiation could be observed from phylogenetic analysis, however, considering the low coverage of countries or geographical regions, this was understandable.

The selection pressure working on CP and MP genes from the RBDV populations was studied based on the $d_S/d_N$ ratio calculated from the average number of non-synonymous substitutions per non-synonymous site ($d_S$), and the average number of synonymous substitutions per synonymous site ($d_N$) using algorithms implemented in DnapSp v6 (Rozas et al. 2017). Nucleotide diversity of CP and MP sequences was similar, but in both cases lower in grapevine (GR) (Table 3). The ratio

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**Fig. 2** Evolutionary analysis of RBDV sequences originating from *Rubus* spp. and grapevine (*Vitis* sp.), based on the nucleotide sequences of the RNA2 (2124 nt, 2248 positions). Horizontal lines are in proportion to the number of substitutions per site. Numbers at branch nodes represent bootstrap support of 1000 replicates, but only values higher than 50% are shown. Sequences in bold were obtained during this study.
\( \frac{d_N}{d_S} \) was significantly below 1.0 indicating a strong purifying (stabilizing) selection acting on both CP and MP genes. Cumulative behaviour plots (Fig. 4) for synonymous and non-synonymous substitutions along the coding regions were constructed using the ‘xyplot’ option of

**Fig. 3** Predicted relationships between RBDV sequences originating from *Rubus* spp. and grapevine (*Vitis* sp.), based on the nucleotide sequences of CP gene (822 nt). Horizontal lines are in proportion to the number of nucleotide differences between branch nodes. Numbers at branch nodes represent bootstrap support of 1000 replicates, but only values higher than 50% are shown. Sequences in bold were obtained during this study.
| GenBank Accession Number | Isolate name (RNA/gene) | Country     | Host          | Reference                |
|--------------------------|-------------------------|-------------|---------------|--------------------------|
| FR687358                 | SE3 (RNA2)              | Sweden      | red raspberry | Valasevich et al. 2011   |
| FR687357                 | BY22 (RNA2)             | Belarus     | red raspberry | Valasevich et al. 2011   |
| FR687356                 | BY8 (RNA2)              | Belarus     | red raspberry | Valasevich et al. 2011   |
| FR687355                 | BY3 (RNA2)              | Belarus     | red raspberry | Valasevich et al. 2011   |
| FR687354                 | BY1 (RNA2)              | Belarus     | red raspberry | Valasevich et al. 2011   |
| NC_003740                | R15 (RNA2)              | UK          | red raspberry | Natsukai et al. 1991     |
| KJ007640                 | Ec_Az (RNA2)            | Ecuador     | R. glaucus    | Quito-Avila et al. 2014  |
| AB948215                 | J1 (RNA2)               | Japan       | Red raspberry | Isogai et al. 2014 (unpublished) |
| EU796090                 | CmGR-2 (RNA2)           | Slovenia    | C. murale     | Mavrič Pleško et al. 2009|
| EU796089                 | CmRR-1 (RNA2)           | Slovenia    | C. murale     | Mavrič Pleško et al. 2009|
| EU796088                 | RR-1 (RNA2)             | Slovenia    | red raspberry | Mavrič Pleško et al. 2009|
| EU796087                 | GR-2 (RNA2)             | Slovenia    | grapevine     | Mavrič Pleško et al. 2009|
| EU796086                 | GR-4 (RNA2)             | Slovenia    | grapevine     | Mavrič Pleško et al. 2009|
| EU796085                 | GR-6 (RNA2)             | Slovenia    | grapevine     | Mavrič Pleško et al. 2009|
| DQ120126                 | R.multi. (RNA2)         | China       | R. multibracteatus | Chamberlain et al. 2003 |
| KY417868                 | RR-2 (RNA2)             | Slovenia    | red raspberry | This study               |
| KY417869                 | RR-3 (RNA2)             | Slovenia    | red raspberry | This study               |
| KY417870                 | RR-5 (RNA2)             | Slovenia    | red raspberry | This study               |
| KY417871                 | RR-8 (RNA2)             | Slovenia    | red raspberry | This study               |
| KY417872                 | GR-7 (RNA2)             | Slovenia    | grapevine     | This study               |
| KY417880                 | GR-8 (RNA2)             | Slovenia    | grapevine     | This study               |
| KY417881                 | GR-9 (RNA2)             | Slovenia    | grapevine     | This study               |
| KY417873                 | GR-10 (RNA2)            | Slovenia    | grapevine     | This study               |
| KY417874                 | GR-11 (RNA2)            | Slovenia    | grapevine     | This study               |
| KY417875                 | GR-12 (RNA2)            | Slovenia    | grapevine     | This study               |
| KY417876                 | GR-13 (RNA2)            | Slovenia    | grapevine     | This study               |
| D01052                   | R15 (CP)                | UK          | C. quinoa     | Mayo et al. 1991         |
| AB698501                 | J3 (CP)                 | Japan       | red raspberry | Isogai et al. 2012       |
| AB698500                 | J2 (CP)                 | Japan       | red raspberry | Isogai et al. 2012       |
| AB698499                 | J1 (CP)                 | Japan       | red raspberry | Isogai et al. 2012       |
| AY894679                 | CP Z13-b (CP)           | Finland     | red raspberry | Wang et al. 2008         |
| AY894678                 | CP Z13-a (CP)           | Finland     | red raspberry | Wang et al. 2008         |
| AF259798                 | Can-S (CP)              | Canada      | N. benthamiana| Jones et al. 2000        |
| AF259796                 | D1 (CP)                 | Scotland    | C. quinoa     | Jones et al. 2000        |
| AF259795                 | D200 (CP)               | Scotland    | red raspberry | Jones et al. 2000        |
| KY417877                 | RR-4 (CP)               | Slovenia    | red raspberry | This study               |
| KY417878                 | RR-6 (CP)               | Slovenia    | red raspberry | This study               |
| KY417879                 | RR-7 (CP)               | Slovenia    | red raspberry | This study               |
| MF446640                 | RBDV-HUSZHU (CP)        | Hungary     | grapevine     | Czotter et al. 2018      |
| KY308191                 | EB-42 (CP)              | Argentina   | red raspberry | Dal Zotto et al. 2017    |
| KX838924                 | 3124-03D1 (RNA2)        | Canada      | blackcurrant  | James and Phelan 2017    |
| KY3999999                | Oregon (RNA2)           | USA         | blackcurrant  | Thekke-Veetil et al. 2017|
SNAP available on the HIV database website (www.hiv.lanl.gov; Korber 2000). They indicated that non-synonymous substitutions (red line) are distributed equally throughout the length of the MP (Fig. 4a) and CP (Fig. 4b) coding region. Generally, the number of synonymous substitutions over non-synonymous substitutions is greater in protein-coding regions. However, in the case of MP (Fig. 4a), the synonymous substitutions showed a biphasic distribution with its rate being lower than the rate of non-synonymous in the codon region of 1 to 106 codons. Both GR and RU sequences contributed to observed biphasic distribution/phenomenon (data not shown). However, a few codons were found to be under positive selection.

Testing for selective pressures operating on MP and CP genes was performed using an online tool Datamonkey Adaptive Evolution Server (Delport et al. 2010). The analyses showed that 0.16–0.21% of codons were under a negative (purifying) selection. The FEL analysis (Fixed Effects Likelihood) revealed a very small proportion of codons under positive selection in MP gene (0.006%) and none in CP gene (Table 4). In addition, we used MEME (Mixed Effects Model of Evolution) as it was shown to have greater resolving

Table 3 Estimates of mean codon-based evolutionary diversity

| Gene | Group | n  | No. codons | $P_i(s)$ | $P_i(a)$ | $d_{s}/d_{a}$ |
|------|-------|----|------------|----------|----------|---------------|
| MP   | All   | 26 | 358        | 0.097    | 0.017    | 0.193         |
|      | RU    | 15 | 358        | 0.097    | 0.013    |               |
|      | GR    | 11 | 358        | 0.021    | 0.007    |               |
| CP   | All   | 39 | 274        | 0.128    | 0.012    | 0.086         |
|      | RU    | 27 | 274        | 0.115    | 0.010    |               |
|      | GR    | 12 | 274        | 0.029    | 0.005    |               |

RU Rubus isolates, GR grapevine isolates, n - number of analyzed sequences in the group, $P_i(s)$ - number of synonymous substitutions per site, $P_i(a)$ - number of non-synonymous substitutions per site

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power than FEL (Murrell et al. 2012). Test revealed that five codons for MP and two for CP gene could be under a positive selection, which represents 0.014–0.007% of codons in total. The majority of codons are therefore under neutral selection, which agrees with the calculated dN/dS ratio (Table 3). The sites of episodic diversifying selection that could be mapped to specific lineages are summarized in Table 4. The positively selected sites were found to be present in RBDV lineages originating from *Rubus*, and less from grapevine. A rapid screening for recombination events was performed using GARD (Kosakovsky Pond et al. 2006), a recombination detection method implemented in Datamonkey server (Delpor et al. 2010) and RDP4 program using default parameters (Martin et al. 2015). We found no evidence for recombination events.

Sequence divergence, genetic differentiation and gene flow were estimated by algorithms implemented in DnaSP v6 (Rozas et al. 2017). To estimate genetic differentiation, four subpopulation groups were created: (1) GR group with sequences from grapevine, (2) RU group with sequences from *Rubus*, (3) RU-SLO with Slovenian *Rubus* sequences only, and (4) RU-OTH group with *Rubus* sequences not originating from Slovenia. Genetic differentiation between groups was estimated with statistics Κ ست*, Z*, Hست, and Sنم subjected to permutation tests (Hudson et al. 1992; Hudson 2000). The DnaSP tool was used also for estimating gene flow (FST) between populations (Hudson et al. 1992). The comparison of GR and RU groups revealed strong genetic differences in both CP and MP genes, as evidenced by statistically significant values of all statistics for detecting genetic differentiation (Table 5). Hudson’s Sنم indicated evidence for differentiation between GR and RU, and also between Slovenian *Rubus* and other *Rubus* isolates. Hudson’s FST also indicated the presence of inter-population diversity between above-mentioned groups. The FST value of 0.371 for CP group RU-SLO vs. RU-OTH is close to the threshold value of 0.33 (Rozas et al. 2003). This might indicate more frequent gene flow between population groups RU-SLO and RU-OTH as opposed to infrequent gene flow among all other groups. However, similar situation was not detected for MP gene.

In conclusion, our survey confirmed the presence of RBDV in grapevine in all wine-growing regions of

### Table 4  Positively selected sites in MP and CP genes estimated by FEL and MEME models

| RNA2  | Fixed effects likelihood model (FEL) | Mixed effects model of evolution (MEME) |
|-------|-------------------------------------|----------------------------------------|
| ORF   | No. codons | No. negatively selected sites | No. positively selected sites | Sites with episodic diversifying selection | Lineage specific codon diversification |
| MP    | 358        | 59 (0.16%)                | 2 (0.006%)                  | 5 (0.014%)         | 12 (RR2) (DQ120126); 17 (FR687357); 32 (GR9); 257 (DQ120126) (FR687358); 333 (EU796086) (NC_003740); |
| CP    | 274        | 58 (0.21%)                | 0                          | 2 (0.007%)         | 63 (RR2, RR5, RR6, AF259798, KJ007640); 78 (AF259796, D01052, FR687355) |

### Table 5  Genetic differentiation and gene flow estimation between subgroups of RBDV, based on MP and CP gene sequences

| Gene | Comparison/sub-population | \( \pi_{\text{tot}} \) | \( dN/dS \) | \( \kappa_{\text{st}*} \) | \( Z* \) | \( H_{\text{st}} \) | \( S_{\text{nm}} \) | \( F_{\text{ST}} \) |
|------|--------------------------|-----------------|-----------|-----------------|-------|-----------|-----------|-------|
| MP   | GR vs. RU               | 0.028           | 0.193     | 0.204***        | 3.995*** | 0.019*   | 1.000***  | 0.657  |
|      | RU-SLO vs. RU-OTH       | 0.018           | 0.123     | 0.276**         | 3.092*** | 0.077**  | 0.857*    | 0.434  |
| CP   | GR vs. RU               | 0.039           | 0.086     | 0.131***        | 4.968*** | 0.007*   | 1.000***  | 0.538  |
|      | RU-SLO vs. RU-OTH       | 0.028           | 0.077     | 0.109***        | 4.370*** | 0.024**  | 0.808**   | 0.371  |

* \( \pi_{\text{tot}} \): total estimate of nucleotide diversity between subgroups; \( dN/dS \): indicator of selective pressure operating on protein-coding genes (1 – neutral; >1 – positive; <1 – purifying); \( \kappa_{\text{st}*} \), \( Z* \), \( H_{\text{st}} \), \( S_{\text{nn}} \) – statistics with permutation tests for detecting genetic differentiation between subpopulations (*, \( P < 0.05 \); **, \( P < 0.01 \); ***, \( P < 0.001 \)); \( F_{\text{ST}} \) – Wright’s fixation index for quantification of the genetic differentiation (\( F_{\text{ST}} > 0.33 \) suggests infrequent gene flow)
Slovenia with the highest incidence in Podravje and the lowest in Primorska. Several economically important cultivars were found to be infected. The knowledge about RBDV infection in grapevine is still very limited. It has been reported from Slovenia, Hungary and Serbia (Mavrić Pleško et al. 2009; Mavrić Pleško et al. 2012; Czotter et al. 2018; Jevremović and Paunović 2011). However, we would expect that its distribution is wider than currently known. The present study is an important contribution to the knowledge about RBDV variability, especially for grapevine isolates. The study considerably raised the number of available sequences in the databases which may help to improve the available molecular tests for RBDV detection in research and diagnostics. The phylogenetic analysis of RBDV sequences confirmed the host-based differentiation of RBDV isolates. Further differentiation into subclades was observed within grapevine and within Rubus isolates. The data indicate the possible greater variability of RBDV than previously thought. No recombination events were detected within CP and MP genes.

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Compliance with ethical standards

The authors declare that no human participants or animals were involved in this research.

Conflict of interest The authors declare that they have no conflict of interest.

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References

Barbara, D. J., Morton, A., & Knight, V. H. (2001). Distribution of raspberry bushy dwarf virus genotypes in commercial raspberries in England and Wales. Acta Horticulturae, 551, 23–26.

Chamberlain, C. J., Kraus, J., Kohnen, P. D., Finn, C. E., & Martin, R. R. (2003). First report of raspberry bushy dwarf virus in Rubus multibracteatus from China. Plant Disease, 87, 603.

Czotter, N., Molnar, J., Szabo, E., Demian, E., Kontra, L., Baksa, I., Szititya, G., Kocsis, L., Deak, T., Bisztray, G., Tusnady, G. E., Burgyan, J. & Varallyay, E. (2018). NGS of virus-derived small RNAs as a diagnostic method used to determine viromes of Hungarian vineyards. Frontiers in Microbiology, 9, article 122. https://doi.org/10.3389/fmicb.2018.00122.

Dal Zotto, A., Cardozo, A., Cabrera Mederos, D., Nome, C., Giolitti, F., & Cobelo, C. (2017). First report of raspberry bushy dwarf virus infecting raspberry in Argentina. Journal of Plant Pathology, 99(2), 539.

Delpont, W., Poon, A. F. Y., Frost, S. D. W., & Kosakovský Pond, S. L. (2010). Datamonkey 2010: A suite of phylogenetic analysis tools for evolutionary biology. Bioinformatics, 26(19), 2455–2457.

Derrick, K. S., Beretta, M. J., & Barthe, G. A. (2006). Detection of an Idaeoivirus in Citrus with implications as to the cause of citrus blight. Proceedings of the Florida State Horticultural Society, 119, 69–72.

Dulić-Marković, I., & Ranković, M. (1992). The occurrence of raspberry bushy dwarf virus in Willamette raspberry in Yugoslavia. Acta Horticulturae, 308, 109–112.

Hudson, R. R. (2000). A new statistic for detecting genetic differentiation. Genetics, 155, 2011–2014.

Hudson, R. R., Boos, D. D., & Kaplan, N. L. (1992). A statistical test for detecting geographic subdivision. Molecular and Biological Evolution, 9(1), 138–151.

Isogai, M., Yoshida, M., Imanishi, H., & Yoshikawa, N. (2012). First report of raspberry yellows disease caused by raspberry bushy dwarf virus in Japan. Journal of General Plant Pathology, 78, 360–363.

James, D., & Phelan, J. (2017). Complete genome sequence and analysis of blackcurrant leaf chlorosis associated virus, a new member of the genus Idaeovirus. Archives of Virology, 162(6), 1705–1709.

Jevremović, D., & Paunović, S. (2011). Raspberry bushy dwarf virus – A grapevine pathogen in Serbia. Pesticides and Phytotheraphy (Belgrade), 26(1), 55–60.

Jones, A. T., Murant, A. F., & Jennings, D. L. (1982). Association of raspberry bushy dwarf virus with raspberry yellows disease; reaction of Rubus species and cultivars, and the inheritance of resistance. Annals of Applied Biology, 100, 135–147.

Jones, A. T., McGavin, W. J., Mayo, M. A., Angel-Diaz, J. E., Kärenlampi, S. O., & Kokko, H. (2000). Comparisons of some properties of two laboratory variants of Raspberry bushy dwarf virus (RBDV) with those of three previously characterised RBDV isolates. European Journal of Plant Pathology, 106, 623–632.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjes, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647–1649.

Kokko, H., Lemmetty, A., Haimi, P., & Kärenlampi, S. (1996). New host for raspberry bushy dwarf virus: Arctic bramble (Rubus arcticus). European Journal of Plant Pathology, 102, 713–717.

Kooyman, P., Engelbrecht, D. J., & Kasdorf, G. G. F. (1982). Isolation of raspberry bushy dwarf virus from youngberry in South Africa. Acta Horticulturae, 129, 59–62.

Korber, B. (2000). HIV signature and sequence variation analysis. In A. G. Rodrigo & G. H. Learn (Eds.), Computational analysis of HIV molecular sequences (pp. 55–72). Dordrecht: Kluwer Academic Publishers.

Kosakovsky Pond, S. L., Posada, D., Gravenor, M. B., Woelk, C. H., & Frost, S. D. W. (2006). Automated phylogenetic detection of recombination using a genetic algorithm. Molecular Biology and Evolution, 23(5), 1981–1901.

Kumar, S., Stecher, G., Li, M., Kayaz, C., & Tamura, K. (2018). MEGAX: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35(6), 1547–1549.

Martin, R. R., MacFarlane, S., Sabanadzovic, S., Quito, D., Poudel, B., & Tzanetakis, I. E. (2013). Viruses and virus diseases of Rubus. Plant Disease, 97(2), 168–182.

Martin, D. P., Murrell, B., Golden, M., Khoosal, A., & Muhire, B. (2015). RDP4: Detection and analysis of recombination patterns in virus genomes. Virus Evolution. https://doi.org/10.1093/ve/vev003.

Matus, J. T., Medina, C., & Arce-Johnson, P. (2008). Virus incidence in raspberries, blackberries and red currant commercial plantings of central and South Chile. Acta Horticulturae, 777, 361–366.

Mavić Pleško, I., Viršček Marn, M., Širca, S., & Urek, G. (2009). Biological, serological and molecular characterization of raspberry bushy dwarf virus from grapevine and its detection in the nematode Longidorus juvenilis. European Journal of Plant Pathology, 123(3), 261–268.

Mavić Pleško, I., Viršček Marn, M., Nyerges, K., & Lazar, J. (2012). First report of raspberry bushy dwarf virus infecting grapevine in Hungary. Plant Disease, 96(10), 1582.

Mavić, I., Viršček Marn, M., Koron, D., & Želzina, I. (2003). First report of raspberry bushy dwarf virus on red raspberry and grapevine in Slovenia. Plant Disease, 87(9), 1148.

Mavić, I., Viršček Marn, M., & Koron, D. (2004). Detection of Raspberry bushy dwarf virus in some raspberry cultivars in Slovenia. Acta Horticulturae, 656, 155–158.

Mayo, M. A., Jolly, C. A., Murant, A. F., & Raschke, J. H. (1991). Nucleotide sequence of Raspberry bushy dwarf virus RNA-3. Journal of General Virology, 72, 469–472.

Murrell, B., Wertheim, J. O., Moolo, S., Weighill, T., Scheffler, K., & Kosakovsky, S. L. (2012). Detecting individual sites subject to episodic diversifying selection. PLoS Genetics, 8(7), e1002764.

Natsuaki, T., Mayo, M. A., Jolly, C. A., & Murant, A. F. (1991). Nucleotide sequence of raspberry bushy dwarf virus RNA-2: A bicistronic component of a bipartite genome. Journal of General Virology, 72(9), 2183–2189.

Navarro, B., Loconsole, G., Giampetruzzi, A., Aboughanem-Sabanadzovic, N., Ragozzino, A., Ragozzino, E., & Di Serio, F. (2016). Identification and characterization of privet leaf blotch-associated virus, a novel Idaeovirus. Molecular Plant Pathology. https://doi.org/10.1111/mpp.12450.

Quito-Avila, D. F., Ibarra, M. A., Alvarez, R. A., Espinoza, L., Ratti, M. F., Peralta, E. L., & Martin, R. R. (2013). First report of raspberry bushy dwarf virus in andean blackberry (Rubus glaucus) in Central Ecuador. Plant Disease, 97(7), 1003.

Quito-Avila, D. F., Lightle, D., & Martin, R. R. (2014). Effect of raspberry bushy dwarf virus, raspberry leaf mottle virus, and raspberry latent virus on plant growth and fruit crumbliness in ‘Meeker’ red raspberry. Plant Disease, 98(2), 176–183.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guiaro-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large datasets. Molecular and Biological Evolution, 34, 3299–3302.

Spak, J., & Kubelkova, D. (2000). Epidemiology of raspberry bushy dwarf virus in the Czech Republic. Journal of Phytopathology, 148, 371–377.

Strik, B., & Martin, R. R. (2003). Impact of raspberry bushy dwarf virus on ‘Marion’ blackberry. Plant Disease, 87, 294–296.

Thekke-Veetil, T., Ho, T., Postman, J. D., & Tzanetakis, I. E. (2017). Characterization and detection of a novel idaeovirus infecting blackcurrant. European Journal of Plant Pathology. https://doi.org/10.1007/s10658-017-1211-z.

Valasevich, N., Kukharchyk, N., & Kvarnheden, A. (2011). Molecular characterization of raspberry bushy dwarf virus isolates from Sweden and Belarus. Archives of Virology, 156, 369–374.

Wall, R. E., & Shamoun, S. F. (1990). Diseases of Rubus parviflorus in British Columbia. Canadian Plant Disease Survey, 70(2), 133–135.

Wang, Q., Cuellar, W. J. M., Rajamaki, M.-L., Hirata, Y., & Valkonen, J. P. T. (2008). Combined thermotherapy and cryotherapy for efficient virus eradication: relation of virus degradation in shoot tips. Molecular Plant Pathology, 9(2), 237–250.

Wood, G. A. (1995). Further investigations of raspberry bushy dwarf virus. New Zealand Journal of Crop and Horticultural Science, 23(3), 273–281.

Ziegler, A., Natsuaki, T., Mayo, M. A., Jolly, C. A., & Murant, A. F. (1992). The nucleotide sequence of RNA-1 of raspberry bushy dwarf virus. Journal of General Virology, 73, 3213–3218.