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ORIGINAL ARTICLE

Incidence and genetic diversity of raspberry bushy dwarf virus and raspberry leaf blotch virus isolates in red raspberry in Ukraine

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Abstract

The objective of this study was to assess the prevalence and genetic diversity of raspberry bushy dwarf virus (RBDV) and raspberry leaf blotch virus (RLBV) in Ukrainian raspberry plantations. A survey of viral symptoms, including yellow leaf blotch, leaf distortion, mottling, and crumbly fruits, was conducted across several regions. Crumbly fruits were linked to RBDV infection, while yellow leaf blotch and leaf distortion were associated with RLBV and the presence of eriophyid mites, which serve as RLBV vectors. To detect the presence of these viruses, 195 symptomatic and asymptomatic leaf samples were collected from cultivated raspberry fields and tested using RT-PCR. The overall infection rate was 33.3%, with RBDV detected in 29.2% of the samples and RLBV in 4%. Comparison of sequences of the Ukrainian RBDV isolates showed high nucleotide and amino acid identity and grouped them with isolates from Belarus, Sweden, and Kazakhstan. RLBV isolates from Ukraine displayed notable genetic variability, clustering with isolates from Finland, Serbia, and Slovakia. These findings highlight a high level of virus infections in raspberry plantations in Ukraine. Despite the obligatory certification of planting material, RBDV remains a significant threat to raspberry farming. Furthermore, the emergence and spread of RLBV in Ukraine could further worsen the situation.

Keywords: phylogenetic analysis, *Rubus idaeus* L., raspberry bushy dwarf virus (RBDV), raspberry leaf blotch virus (RLBV), RT-PCR

Introduction

Raspberry (*Rubus idaeus* L.) is one of the most important berry crops in Ukraine. Its commercial plantations cover up to 5,000 hectares, with an annual harvest of fresh berries reaching 65-68 thousand tons (UKRSTAT 2022). Currently, more than 40 viral and virus-like pathogens are known to infect *Rubus* species and can lead to significant crop losses (McGavin and MacFarlane 2010).

Some of the most common raspberry diseases are leaf yellowing, dwarfing, and crumbly fruit, of which the viral nature was confirmed in the 1950-60s in the USA (Cadman 1961). These symptoms were associated with raspberry bushy dwarf virus (RBDV), although it was later shown that the severity of such symptoms is significantly higher in mixed infections with raspberry leaf mottle virus, rubus yellow net virus, and raspberry latent virus (Martin 2013; Quito-Avila *et al*. 2013).

RBDV often occurs in raspberry and blackberry, and has been found in grapevine and cherry (Mavrič *et al*. 2003; Martin 2013; Çağlayan *et al*. 2023a). It is present on *Rubus* spp. in all regions where they are

cultivated, including Europe, Asia, New Zealand, North and South America, and South Africa (Mavrič *et al*. 2003; Valasevich *et al*. 2011; Isogai *et al*. 2012; Quito-Avila *et al*. 2013; Dal Zotto *et al*. 2017).

A major challenge posed by this virus is its ability to be transmitted through pollen and seeds from an infected plant to a healthy one, complicating efforts to control its spread in cultivated raspberry plantations. Nearly 77% of seedlings grown from seeds of infected red raspberries were also infected by RBDV (Jones *et al*. 1996). It has also been found that the viability of pollen infected with this virus remains quite high. During pollination, the virus affects the germination rate of pollen tubes (Sproge and Strautina 2020).

Currently, several groups of RBDV isolates are known: S (the most numerous group), RB (isolates that can infect hybrids and cultivars resistant to S-isolates) and B isolates (serologically distinct and detected only in *R. occidentalis*) (Jones *et al.* 1996). The spread of RBDV can be controlled by cultivating raspberry cultivars with resistance genes, particularly the Bu gene, a single-dominant gene originally identified in *R. idaeus* "Glen Glova" (Jones *et al*. 1982). Efforts to develop markers for Bu have led to several promising molecular markers associated with two N-like NB-LRR genes (N1 and N2), which are homologous to the TMV-resistance gene found in tobacco. However, it is still unclear as to which N-genes the Bu gene aligns (Ward *et al*. 2012; Stephens *et al.* 2016). While the Bu gene provides resistance to S-isolates of RBDV, resistance-breaking isolates continue to present a challenge for raspberry cultivation, underlining the need for ongoing breeding to expand resistance in new cultivars (Hall *et al*. 2009; Stephens *et al*. 2016).

RBDV belongs to the species *Idaeovirus rubi,* which is one of the two species in the genus *Idaeovirus*, and the family *Mayoviridae* (Schoch *et al.* 2020). The RBDV genome consists of two single-stranded sense RNA molecules: RNA1 (5.5 kb) and RNA2 (2.2 kb), which is comprised of a subgenomic RNA (sgRNA3) (1 kb). RNA1 encodes a 190 kDa polyprotein involving methyltransferase, helicase and polymerase domains, and 1b protein. RNA2 encodes a 39 kDa movement protein (MP) and a 30 kDa coat protein (CP). The infection process crucially depends on CP, expressed from sgRNA3, and facilitated by 1b protein that acts like a RNA silencing suppressor (Isogai *et al.* 2019).

Another common raspberry disease is leaf blotch, caused by the raspberry leaf blotch virus (RLBV). It was first described in Scotland in 2011 (McGavin *et al*. 2012). After that, RLBV has also been discovered in European countries with significant raspberry production, such as Bosnia and Herzegovina, Bulgaria, Finland, Montenegro, Poland, Serbia, Slovakia, the Czech Republic, and Norway (Dong *et al.* 2016; Cieslińska *et al.* 2014; Mavrič *et al.* 2014; Zindović *et al.* 2015; Jevremović *et al.* 2019; Delić *et al.* 2020; Koloniuk *et al.* 2023), as well as in Kazakhstan (Kerimbek *et al.* 2022). RLBV is known to infect red raspberry (*R. idaeus*), tayberry (*R. fruticosus* × *R. idaeus*), and *R. odoratus* (Dong *et al.* 2016). It causes symptoms such as leaf curling and twisting, distorted leaf shape, chlorotic spots on leaves, reduced plant growth due to damage to the apical part of the shoot, and deterioration in fruit quality (McGavin *et al.* 2012). RLBV is transmitted by the mite *Phyllocoptes gracilis* (*Eriophyidae*), which was previously associated with leaf blotch disorder (RLBD) in Great Britain (McGavin *et al*. 2012).

RLBV belongs to the species *Emaravirus idaeobati* genus *Emaravirus* and the family *Fimoviridae* (Schoch *et al.* 2020). RLBV has a segmented multipartite genome of 17,410 nt in length, consisting of eight linear negative sense RNA fragments (Lu *et al*. 2015). RNA1 (7.1 kb) encodes a putative RNA-dependent RNA polymerase, RNA2 (2.1 kb) encodes two glycoproteins, RNA3 (1.4 kb) encodes a nucleocapsid protein (NP) and RNA4 (1.7 kb) encodes a movement protein, while the functions of RNA5–8, which encode several proteins, are not yet clear (McGavin *et al.* 2012; Yu *et al.* 2013; Lu *et al*. 2015).

Symptoms of leaf blotch, dwarfing plants, and crumbly fruit in raspberries have been noted in plantings in Ukraine for many years. However, it was not until recently, that monitoring studies using serological methods were conducted to detect raspberry viruses listed in the EPPO PM 4/10 (2) standard (Tryapitsyna *et al.* 2013; Riaba *et al.* 2019). Varying levels of RBDV, raspberry ringspot virus (RRSV), strawberry latent ringspot virus (SLRV), raspberry vein chlorosis virus (RVCV), tomato black ring spot virus (TBRV), tobacco ringspot virus, cherry leaf roll virus (CLRV), and arabis mosaic virus (ArMV) were detected (Tryapitsyna *et al.* 2013; Riaba *et al.* 2019). The first incidence of red raspberry infected with RLBV in Ukraine was only recently detected in 2021 Pozhylov *et al.* 2021).

Thus, current information on the molecular-genetic characteristics of raspberry viruses in Ukraine is limited. The objective of this study was to investigate the molecular-genetic characteristics and phylogenetic relationships of RBDV and RLBV isolates present in Ukraine. By focusing on the CP gene, the aim of the work was to determine the genetic variability and molecular features of these isolates, and to trace their evolutionary relationships. This analysis will contribute to optimizing diagnostic methods and tracking the origin, spread, and genetic diversity of these viruses.

Materials and Methods

Plant material

Visual inspections were conducted during the spring and summer seasons (2019-2021) to identify symptoms of viral infection in raspberry plantings. Leaf samples were collected from symptomatic plants showing signs of RBDV and RLBV infections, as well as from asymptomatic plants at the same locations. Fifteen raspberry plantations were observed across nine regions: Cherkasy, Chernihiv, Chernivtsi, Kharkiv, Khmelnytskyi, Kyiv, Vinnytsia, Volyn and Zhytomyr (Table 1). In total, samples of 20 raspberry cultivars were tested, including 'Amira', 'Brusvyana', 'Brusylivska', 'Brusylivskyi standard', 'Glen Ample', 'Heracl', 'Himbotop', 'Imara', 'Joan J', 'Kosmichna', 'Kveli', 'Maravila', 'Octavia', 'Perseya', 'Phenomen', 'Polka', 'Sanya', 'Vasylinka', 'Yellow Giant', and 'Zyugana'. A total of 195 collected samples were tested using the RT-PCR method.

RT-PCR

Total RNA extraction was performed using the Genomic DNA Purification Kit (Thermo Scientific, Lithuania), with 75 mg of plant tissue taken for analysis. The quality of the extracted RNA was evaluated using a DeNovix DS-11 spectrophotometer at wavelengths of 260, 260/230, and 260/280 nm. The RNA was stored at –20°C for future use. RBDV identification was conducted using RT-PCR with primers U1-F and L3-R with expected fragment size of 520 bp specific to the part of the CP coding region (Kokko *et al.* 1996). RLBV was identified using RT-PCR with primers 1287-F and 1095-R, which amplify a 570 bp fragment specific to the NP coding region (McGavin *et al.* 2012). Verso 1-Step RT-PCR Kit ReddyMix (Thermo Scientific, USA) was used for RT-PCR following the manufacturer's recommendations. A total of 50 ng of RNA was added to a 20 µl reaction mixture, which contained the following components: 10 µl of 2X 1 Step PCR ReddyMix, 0.4 µl of Verso Enzyme Mix, 1 µl of RT Enhancer, 0.4 µl of forward primer (10 mmol), 0.4 µl of reverse primer (10 mmol), and 6.6 µl of H_2O. Amplification was performed in an Eppendorf Mastercycler Personal (Eppendorf AG, Germany) with the following parameters: 50°C for 15 min, 95°C for 2 min, followed by 40 amplification cycles (95°C for 20 s, 56–58°C for 30 s, 72°C for 15 s), and a final extension at 72°C for 5 min. The amplification products were analyzed by electrophoresis on a 2% agarose gel in TBE buffer, stained with 0.5 µg/ml ethidium bromide, and visualized under UV light.

Sequencing and phylogenetic analysis

The samples selected for sequencing were also tested for the presence of other raspberry viruses and phytoplasmas listed in EPPO standard PM 4/10 (2) (EPPO 2013) to ensure the absence of co-infection (not shown). Analyses were performed using previously published primers (Supplementary file). The PCR products of RBDV and RLBV obtained from samples collected in various regions of Ukraine were sequenced and deposited in the GenBank. The partial sequences of the CP genes for RBDV and RLBV were compared with known sequences using BLAST 2.10.0 software. The percentage of identity for nucleotide (nt) and amino acid (aa) sequences was represented as colored blocks employing the Sequence Demarcation Tool Version 1.2 (SDTv1.2) (Muhire *et al.* 2014). For phylogenetic analysis, 40 RBDV isolates and

Table 1. Raspberry samples tested for RBDV and RLBV by RT-PCR method

Region	No. of tested samples	RBDV	RLBV	No. of localities	Cultivars that tested positive
Cherkasy	6	0	0	1	–
Chernivtsi	11	1	0	1	'Heracl'
Chernihiv	5	0	0	1	–
Kharkiv	12	3	0	1	'Polka'
Khmelnytskyi	8	2	0	1	–
Kyiv	73	31	6	3	'Yellow Giant', 'Perseya', 'Brusvyana', 'Zyugana', 'Vasylinka', 'Imara'
Vinnytsia	38	17	2	4	'Joan J', 'Amira', 'Octavia', 'Kveli'
Volyn	10	0	0	1	–
Zhytomyr	32	3	0	2	'Maravila'
Total	195	57	8	15	

RBDV – raspberry bushy dwarf virus; RLBV – raspberry leaf blotch virus

23 RLBV isolates were downloaded from the GenBank. Multiple sequence alignment was conducted using the Clustal W algorithm within MEGA X (Kumar *et al.* 2018). Phylogenetic trees were constructed using the neighbor-joining (NJ) method (Saitou and Nei 1987) with the Kimura two-parameter model (Kimura 1980). The statistical significance of the results was assessed through bootstrap analysis with 1,000 replications. Potential recombination events were evaluated using RDP4 (Martin *et al.* 2015) and were considered significant if detected by most of the algorithms used.

Results and Discussion

Occurrence of RBDV and RLBV in Ukraine

The most common symptoms observed in the majority of surveyed raspberry plantations were yellow leaf blotch, leaf yellowing, distortion of leaves, mottling, and crumbly fruits (Fig. 1, 2). Crumbly fruit symptoms are most often caused by RBDV, while yellow leaf blotch could be caused by RLBV and eriophyid mite, which is a vector of this virus. Additionally, leaf chlorotic mottling and vein chlorosis were observed in some surveyed areas.

A total of 195 raspberry leaf samples collected in cultivated fields were tested by RT-PCR for the presence of RBDV and RLBV. Testing revealed an overall infection rate of 33.3% (Table 1).

RBDV was detected in 29.2% of raspberry samples, while RLBV was present in 4% of the total tested samples. No coinfection was detected in any samples. During the survey 14% of RBDV-infected plants showed no symptoms. Similar RBDV infection rates have been observed in raspberry plantations in other countries, with 35% of infected samples in Latvia, 22% in Scotland, 28% in Kazakhstan, and 12% in Finland (Chard *et al.* 2001; Pupola *et al.* 2009; Susi *et al.* 2018; Kolchenko *et al.* 2023). Additionally, this virus was found in 22% of the grapevine samples in Slovenia (Mavrič *et al.* 2014). In contrast, RLBV has been reported in 93% of the raspberry samples in Finland, 68.7% in Serbia, and 30% in Bosnia and Herzegovina (Dong *et al.* 2016; Jevremović *et al.* 2019; Delić *et al.* 2020). These high infection rates for RLBV are likely due to studies focusing on samples exhibiting typical symptoms of viral infection and infestation of eriophyid mites. Experiments in raspberry growing countries have shown that increasing mite populations can lead to significant economic losses and facilitate the spread of RLBV, making control of the eriophyid mite in Ukraine an increasingly important issue (Podedworny *et al.* 2024).

Fig. 2. Symptoms of raspberry leaf blotch disease (chlorotic blotches) on leaves of raspberry plants cv. Octavia infected with RLBV

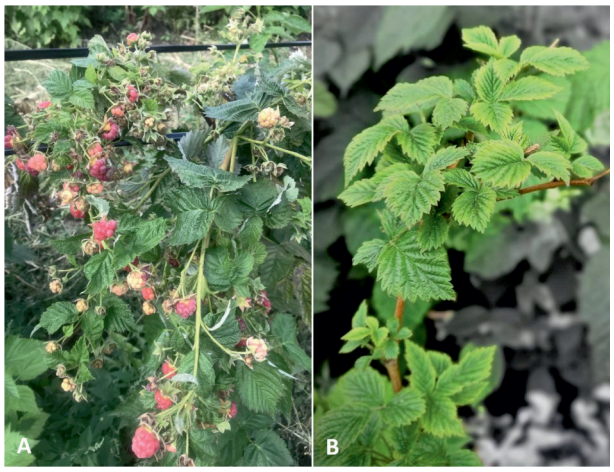

Fig. 1. Symptoms of: A – crumbly fruits of raspberry cv. Joan J infected with RBDV; B –dwarfing of raspberry leaves cv. Polka infected with RBDV

RBDV was detected in six regions of Ukraine (Chernivtsi, Kharkiv, Khmelnytskyi, Kyiv, Vinnytsia, and Zhytomyr), indicating a widespread distribution. The highest infection rates were observed in the Kyiv and Vinnytsia regions, with 42.5% and 44.7% of the analyzed samples, respectively. RLBV was also identified in these regions, with 8.3% of the samples testing positive in Kyiv and 5.3% in Vinnytsia.

The phytovirological status was significantly better in Zhytomyr, with only 9.4% of the samples testing positive for RBDV, and no RLBV detected. In Kharkiv and Khmelnytskyi, 25% of the samples in each region tested positive for RBDV. In Chernivtsi, approximately 9% of the samples were infected with RBDV, while no RLBV was detected. In contrast, plant material collected from cultivated raspberry plantations in Cherkasy,

Volyn, and Chernihiv regions was free of both RBDV and RLBV. Overall, these findings highlight substantial variability in the distribution of RBDV and RLBV among raspberry plants across different regions of Ukraine. The presence of these viruses in certain regions may be influenced by local agricultural practices, environmental factors, and the movement of infected plant material.

Only three out of the 20 surveyed cultivars ('Perseya', 'Sanya', and 'Perlinka') are summer-bearing, as primocane-fruiting cultivars are more popular in commercial plantations in Ukraine due to their higher profitability. However, primocane cultivars have a higher risk of infection with contaminated pollen because of their longer flowering period.

Phylogenetic analysis of RBDV

For further analysis CP gene partial sequences of four RBDV isolates were sequenced: 177UA, 431UA, 87UA, and UA1, respectively, isolated from the cultivars 'Heracl' (Chernivtsi region), 'Joan J' (Vinnytsia region), 'Perseya' (Kyiv region), and 'Polka' (Kharkiv region) (Table 2).

After alignment a 466 nt fragment of the RBDV CP gene was obtained, corresponding to the RNA2 region 1365 to 1831 nt. Ukrainian RBDV isolates had a high amino acid and nucleotide sequence identity: 98.28–99.78% and 98.7–100%, respectively. Isolates from Kharkiv (431UA), Kyiv (UA1) and Vinnytsia (177UA) regions had the highest identity among themselves both in terms of nucleotide and amino acid sequences (Table 3). The isolate from Chernivtsi (87UA) region, which differed by one serine (S) → asparagine (N) substitution at position 30 when comparing amino acid sequences with other Ukrainian isolates, turned out to be more remote (Fig. 3). Additionally, nonsynonymous nucleotide substitutions in the 177UA isolate where a substitution of valine (V) → isoleucine (I) was observed at position 86. In general, a high degree of conservativity was observed for this section of the studied isolates.

In the next stage, sequence alignment of the Ukrainian RBDV isolates was performed with 41 available sequences from the GenBank database. The most divergent RBDV isolate, obtained from *R. multibracteatus* in China (DQ120126) (Chamberlain *et al.* 2003), was used as an outgroup to root the tree.

Among the analyzed RBDV isolates, the nucleotides sequence identity varied from 86.3% to 100%, while aa sequence identity varied from 86.1% to 100%. The mean variability across all isolates was calculated to be 95.5%. The dendrogram (Fig. 4) illustrates the grouping of isolates into two main clusters. Cluster I included isolates from Canada, Ecuador, Finland, Great Britain, Hungary, Kazakhstan, Slovenia, and Turkey, all of which exhibited a wide host range, including red raspberry, wild blackberry, grapevine, and

Table 2. Sequences of RBDV and RLBV isolates analysed in this study

Virus	GenBank accession No	Cultivar	Region	Year	Symptom patterns	Isolates
RBDV	MW457595	Polka	Kharkiv	2020	crumbly fruit, dwarfing, interveinal chlorosis	431 UA
RBDV	MW457594	Perseya	Kyiv	2020	druplet abortion, dwarfing, interveinal chlorosis	UA1
RBDV	OR086926	Heracl	Chernivtsi	2021	deformation, crumbly fruit	87 UA
RBDV	OR086927	Joan J	Vinnytsia	2021	crumbly fruit, dwarfing, interveinal chlorosis	177 UA
RLBV	OR086928	Yellow Giant	Kyiv	2021	mottling, yellow leaf blotches	UA117-RLBV
RLBV	OR086929	Octavia	Vinnytsia	2021	yellow leaf blotches	UA178-RLBV

Table 3. Identity of nucleotide and amino acid sequences of Ukrainian isolates of RBDV

Isolates	177UA*		431UA		UA1	
	NT [%]	AA [%]	NT [%]	AA [%]	NT [%]	AA [%]
431UA	99.78	99.35	–	–	–	–
UA1	99.57	100	99.78	100	–	–
87UA	98.28	98.7	98.47	99.35	98.28	99.35

NT – nucleotide sequence identity; AA – amino acid sequence identity.

*isolate designations represent Ukrainian RBDV isolates obtained from specific cultivars: 177UA ('Heracl'), 431UA ('Joan J'), 87UA ('Perseya'), and UA1 ('Polka')

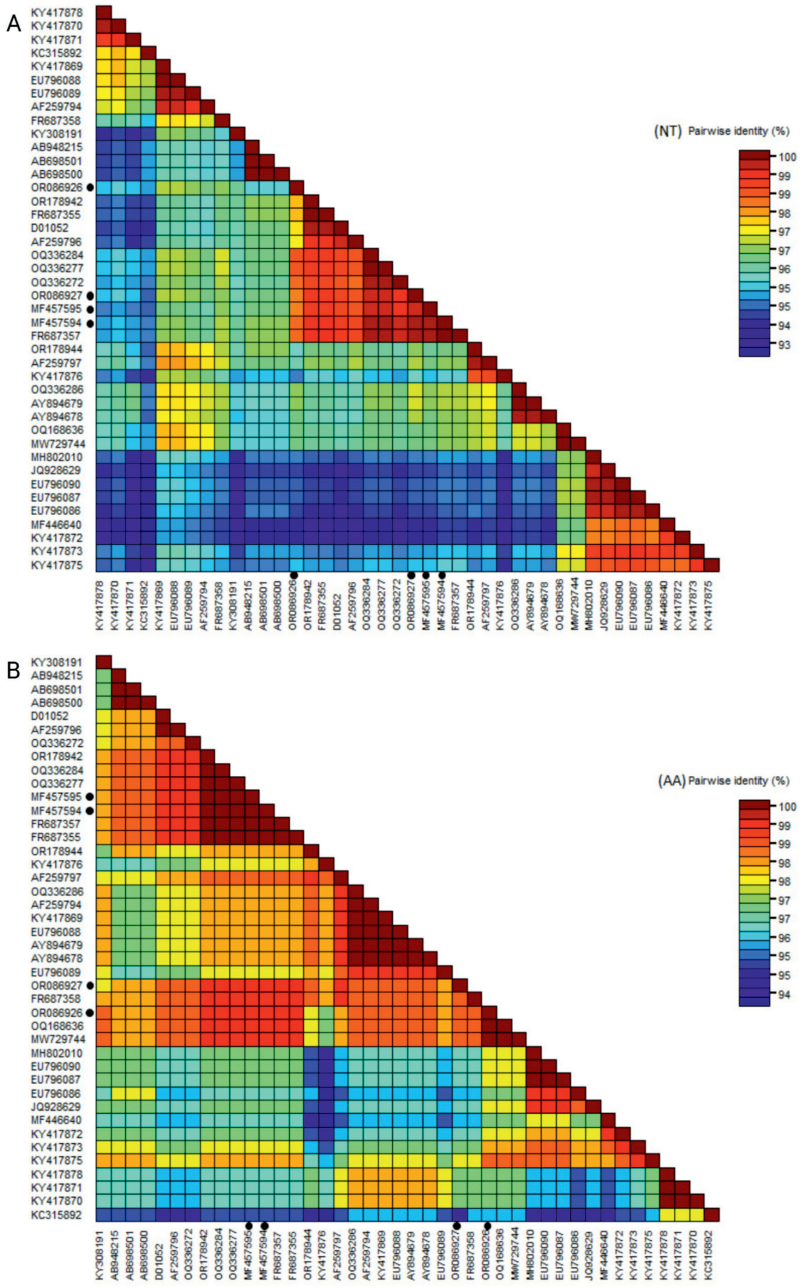

Fig. 3. Two-dimensional visualization of nucleotide – A and amino acids – B partial CP gene sequences identity of 44 RBDV isolates. The matrices were performed using SDTv1.2. Ukrainian isolates marked by black dots

cherry. The identity on the nucleotides level within this cluster was 92.9–100%, while the aa sequence identity varied from 93.3% to 100%. The mean nt and aa sequence variability among these isolates was 96.1% and 96.9%, respectively.

The Ukrainian isolates grouped into cluster II along with isolates from Belarus, Sweden, Japan, the UK, and Kazakhstan, all sharing a common host – *Rubus idaeus* spp. The nucleotide sequence identity within this cluster ranged from 95.5 to 100%, and amino acid identity from 98 to 100%. The mean nucleotide sequence variability for these isolates was 98%, while amino acid variability was 99.1%. Ukrainian isolates were most closely related to those from Belarus. Isolate 431UA showed 100% nt and aa sequence identity with the BY22 isolate, while 177UA, placed in the same clade, had the highest similarity to BY22 with 99.8% nt and 100% aa (Fig. 4). Isolate 87UA, the most distant among Ukrainian isolates, was closest to the Kazakh isolate KZMol3 (nt – 98.7% and aa – 100%).

Overall, isolates from Kazakhstan (KZSelection2-8, KZMol3, KZ3-4) in this cluster showed high similarity to the Ukrainian isolates – 98.7–99.8% for nucleotides and 98.7–100% for amino acids. Meanwhile, the Japanese isolates (from *R. idaeus*) had slightly lower nucleotide sequence identity than the Ukrainian isolates – around 96% nt and 98.7% aa.

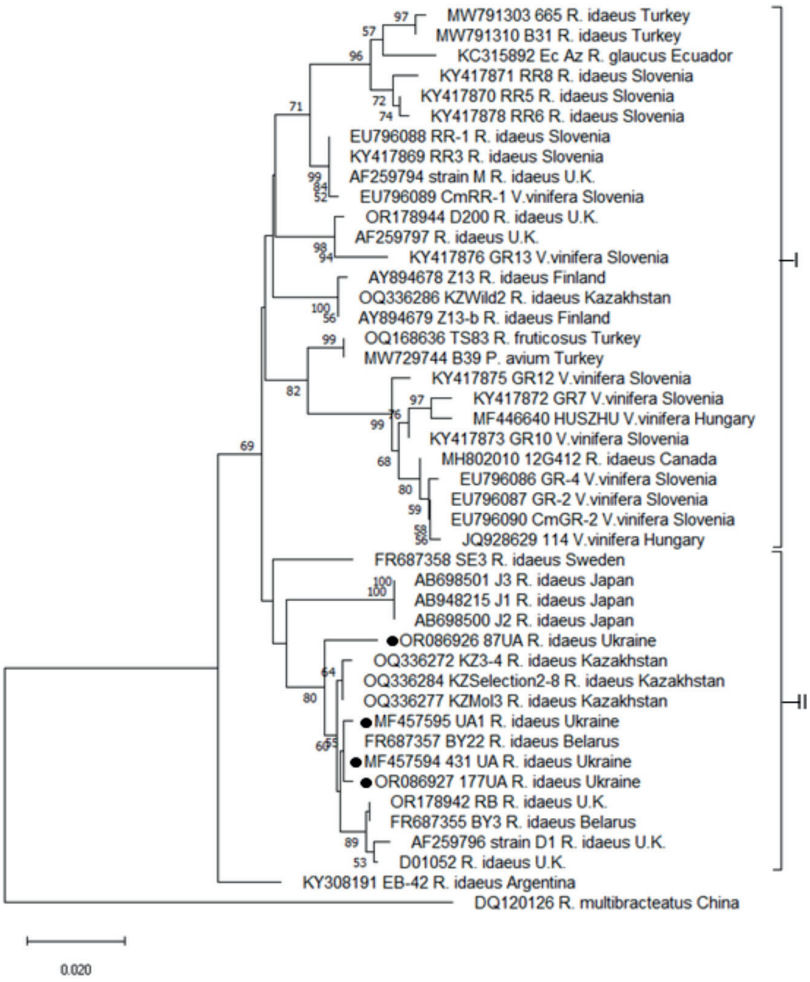

Fig. 4. Phylogenetic tree of 45 RBDV isolates, based on partial sequences of the CP gene using the neighbor-joining algorithm option of MEGA10. Bootstrap analysis of 1000 replicates was performed (bootstrap values >50 are shown). Sequences obtained in this study are marked with black dots

The Ukrainian RBDV isolates likely possess biological properties characteristic of the S-group, as they grouped with the D200 isolate (OR178944) from the UK, showing nucleotide identity of 95.8–96.7% and amino acid identity of 97.4–98.7%. These isolates are unable to infect varieties with resistance controlled by the *Bu* gene (Hall *et al.* 2009). No recombination events were detected among the RBDV isolates in this study, within the investigated part of the genome.

The RBDV genome variability appeared to depend more on the host plant type than its geographical origin, which supports the results from other studies (Valasevich *et al.* 2011; Isogai *et al.* 2012; Mavric *et al.* 2014; Çağlayan *et al.* 2023*a*). A study of RBDV isolates in *Rubus* spp. in Turkey demonstrated a quite low identity level of isolates, even between raspberry and blackberry, highlighting the importance of testing samples using primers targeting different genomic fragments (Çağlayan *et al.* 2023*b*). Low variability of RBDV isolates within a single host could be due to high specialization, vegetative propagation of raspberries, the movement of infected planted material, and transmission by pollen, but absence of cross-pollination between different *Rubus* species (Jones *et al.* 1996). The high identity of Ukrainian and Belarusian isolates may be explained by the close geographical proximity and the possible transmission of infected pollen between plantations. Additionally, the intensive international trade of planting material, propagated vegetatively, poses a direct risk for virus import and may have contributed to this high similarity. RBDV is present in the largest countries exporting raspberry planting material to Ukraine, including the United Kingdom, Italy, and Poland (Ukrainian Ministry of Agriculture 2023). This may explain why isolates from the United Kingdom were found to be almost identical to those from Ukraine. Although RBDV is also present in Poland and Italy (EPPO 2013), tracing phylogenetic connections with these isolates is not possible, as data on their CP gene sequences are absent in the NCBI database.

Phylogenetic analysis of RLBV

Two partial sequences of the NP gene of RLBV isolates UA178-RLBV and UA117-RLBV were obtained from 'Octavia' (Vinnytsia region) and 'Yellow giant' (Kyiv region) cultivars. Both samples showed leaf spot symptoms and had an infestation of the eriophyid mite *Phyllocoptes gracilis*.

The obtained RLBV isolate sequences were 532 nt in length, corresponding to the region from 799 to 1330 nt of RNA3 encoding (NP). The nucleotide sequence identity of the isolates was 95.57%, with 22 nucleotide differences observed. Similarly, the amino acid sequences showed a high identity of 95.8%. Alignment of the NP gene region's amino acid sequences revealed differences at seven positions between isolates UA178 and UA117: glycine (G) → serine (S) (17), threonine (T) → asparagine (N) (44), serine (S) → asparagine (N) (78), phenylalanine (F) → asparagine (N) (79), isoleucine (I) → valine (V) (142), and valine (V) → isoleucine (I) (144, 159). These findings indicate a relatively high level of genetic variability and the presence of nonsynonymous codon substitutions (Fig. 5).

To perform sequence comparison and establish phylogenetic relationships, 23 known RLBV isolates (registered in the NCBI GenBank) that exhibited the highest percentage of sequence overlap with isolates from this study were selected. These isolates also originated from various geographical regions (Fig. 5). For the outgroup, the virus most closely related to RLBV, the Jujube yellow mottle-associated virus from China, was used (Fig. 6).

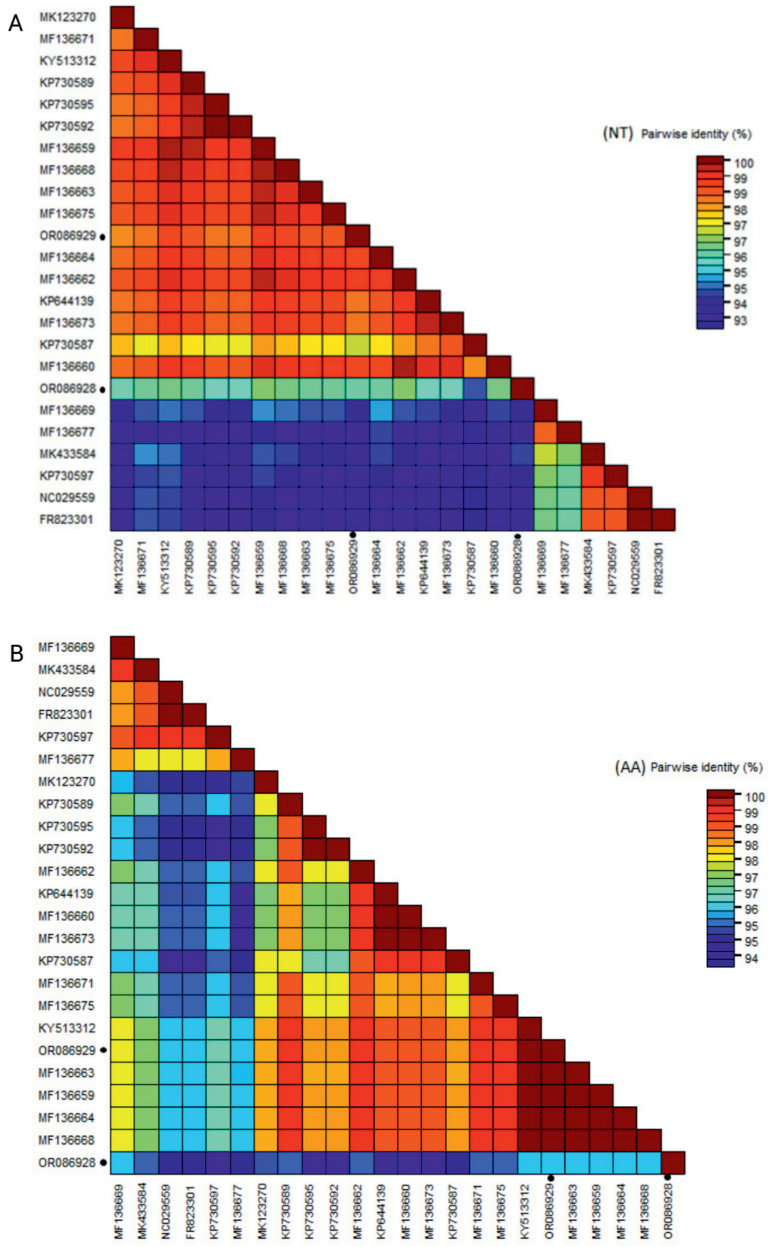

Fig. 5. Two-dimensional visualization of nucleotide – A and amino acids – B partial NP gene sequences identity of 24 RLBV isolates. The matrices were performed using SDTv1.2. Ukrainian isolates marked by black dots

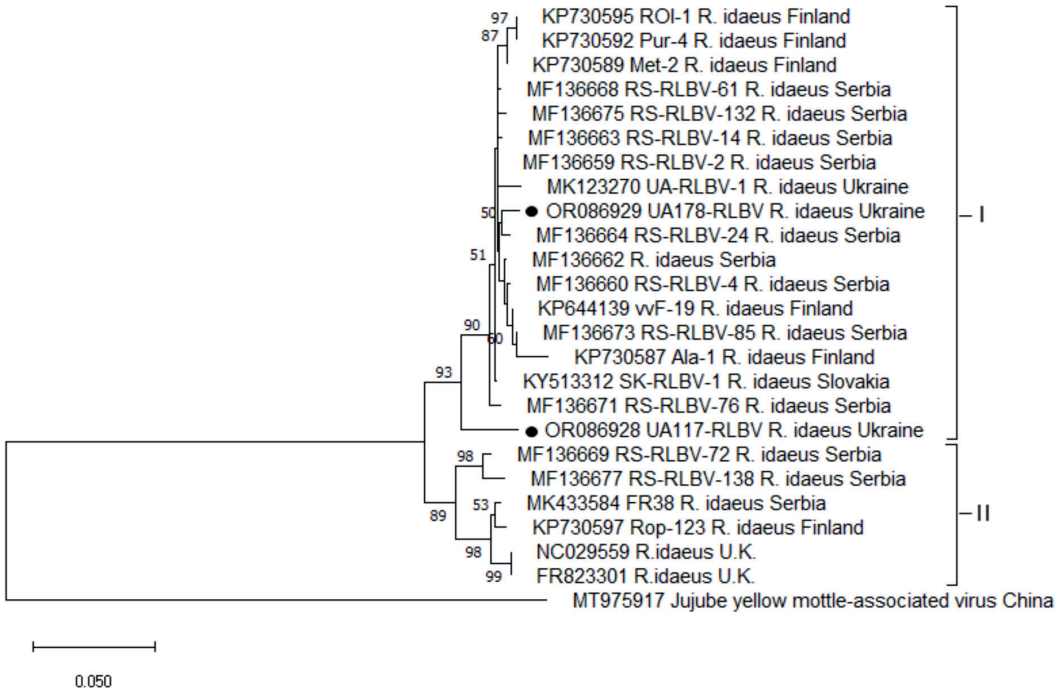

Fig. 6. Phylogenetic tree of 25 RLBV isolates, based on partial sequences of the NP gene using the neighbor-joining algorithm option of MEGA10. Bootstrap analysis of 1000 replicates was performed (bootstrap values >50 are shown). Sequences obtained in this study are marked with black dots

All studied isolates formed two main clusters in the constructed phylogenetic tree. Three Ukrainian isolates grouped in cluster I along with some Finnish, Serbian, and Slovak isolates. While UA178-RLBV and UA-RLBV-1 showed a close relationship with other European isolates, UA117-RLBV was positioned on a separate branch within this cluster. The identity level within cluster I ranged from 93.9% to 99.8% for nt and 94.8% to 100% aa, with mean identity of 98.5% nt and 98.3% aa.

Cluster II included RLBV isolates from the UK, France, and Finland, with nt similarity ranging from 95.8% to 100% and aa sequence similarity from 94.5% to 100%. The mean nt sequence variability in this cluster was 97.4%, while for aa, it was 97.1%.

The nucleotide sequence of the UA178-RLBV isolate showed the highest identity, at 99%, with isolates from Serbia (RS-RLBV-2, RS-RLBV-24, RS-RLBV-64). However, the amino acid sequences of these isolates were 100% identical (Fig. 6). The isolate also had 100% amino acid similarity with the Slovak isolate SK-R -LBV-1, despite nucleotide differences (98.8%).

In contrast, another studied isolate, UA117-RLBV, showed significantly lower identity with European isolates than UA178-RLBV. The highest identity, as with UA178-RLBV, was observed with isolates from Serbia (RS-RLBV-72, RS-RLBV-61) and the Slovak SK-RL -BV-1, with nucleotide identity of 93.4–96% and amino acid identity of 95.8%.

Thus, the genetic variability of RLBV isolates was significantly higher than that of RBDV isolates. Based on these results, it can be concluded that there was considerable genetic diversity among RLBV isolates originating from different geographical regions.

Comparison of the isolates from this study with the only known Ukrainian isolate to date, UA-RLBV-1, revealed a nucleotide sequence identity of 98.2% with UA178-RLBV and 95.1% with UA117-RLBV.

Recombination analysis using RDP4 software detected a recombination event confirmed by three methods (Maxchi, 3Seq, SiScan, $p<0.05$), within the investigated part of the genome. This involved the Finnish isolate Pur-4 as the major parent and the Ukrainian isolate UA178-RLBV as the minor parent. Isolate UA-RLBV-1 likely resulted from recombination with breakpoints at nucleotide positions 855 and 1,283 of RNA3. Recombination within the RLBV population contributes to the virus's genetic diversity, which is associated with adaptation to new hosts, emergence of new viruses, and increased virulence (Moury and Desbiez 2020).

Given the relatively high variability of RLBV isolates in Ukraine, it can be hypothesized that this pathogen was recently introduced from different geographical regions into local agrocenoses. Significant genetic variability has been observed in RLBV populations infecting both cultivated and wild raspberry plants in Finland and Serbia (Dong *et al.* 2016; Jevremović *et al.*

2019), while a high degree of homogeneity was noted in isolates from Bosnia and Herzegovina (Delić *et al.* 2020).

The spread of viral diseases in Ukrainian raspberry plantations requires rigorous protection measures, continuous phytovirological monitoring of planting material, and early detection of viruses through laboratory diagnostics and modern detection methods.

This research represents the first molecular analysis of RBDV isolates and expands knowledge about the molecular characteristics of RLBV isolates in Ukraine. New data are essential for monitoring and management of these viruses in the region. This study also revealed a high occurrence of RBDV and the increasing significance of RLBV that can present major challenges for the raspberry industry in Ukraine. The importance of thorough phytovirological monitoring and control of planting material, as well as the improvement of laboratory methods used for diagnostics are highlighted. Especially as RLBV, unlike RBDV, is still not included in the EPPO certification schemes for virus-free planting material, nor is it listed in Ukrainian state standards for phytosanitary control. This gap in monitoring increases the risk of viruses spreading through infected plant stock and should be revised to avoid potentially extensive movement inside and between countries.

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References

Cadman C. H. 1961. Raspberry viruses and virus diseases in Britain. Horticulture Research 1: 47–61.

Çağlayan K., Gazel M., Roumi V., Lamovsek J., Beber A., Pleško I.M. 2023. Sweet cherry, a new host of Raspberry bushy dwarf virus. Journal of Plant Pathology 105 (3): 307–311. DOI: https://doi.org/10.1007/s42161-022-01257-1

Çağlayan K., Ördek K., Gazel M., Elçi E., Roumi V., Lamovšek J., Mavrič Pleško I. 2023. Incidence and genetic diversity of raspberry bushy dwarf virus (RBDV) in *Rubus* spp. in Turkey. Annals of Applied Biology 183 (3): 209–219. DOI: https://doi.org/10.1111/aab.12847

Chamberlain C.J., Kraus J.E., 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. DOI: https://doi.org/10.1094/PDIS.2003.87.5.603A

Chard J., Irvine S., Roberts A.M.I., Nevison I.M., McGavin W.J., Jones A.T. 2001. Incidence and distribution of Raspberry bushy dwarf virus in commercial red raspberry (*Rubus idaeus*) crops in Scotland. Plant Disease 85 (9): 985–988. DOI: https://doi.org/10.1094/PDIS.2001.85.9.985

Cieslińska M., Tartanus M. 2014. Molecular diversity of Raspberry leaf blotch virus – a new pathogen of Rubus sp. plants in Poland. p.162. In: Book of Abstracts of the 11th Conference of the European Foundation for Plant Pathology. 8–13 September 2014, Publishing House of the University of Agriculture in Krakow, Krakow.

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): 533–543. DOI: https://doi.org/10.4454/jpp.v99i2.3896

Delić D., Radulović M., Vakić M., Sunulahpašić A., Villamor D.E.V., Tzanetakis I.E. 2020. Raspberry leaf blotch emaravirus in Bosnia and Herzegovina: population structure and systemic movement. Molecular Biology Replication 47 (6): 4891–4896. DOI: https://doi.org/10.1007/s11033-020-05560-x

Dong L., Lemmetty A., Latvala S., Samuilova O. 2016. Occurrence and genetic diversity of Raspberry leaf blotch virus (HTS/RT-PCR) infecting cultivated and wild *Rubus* species in Finland. Annals Applied Biology 168 (1): 122–132. DOI: https://doi.org/10.1111/aab.12247

EPPO. 2013. Raspberry bushy dwarf virus. [Distribution map]. Distribution Maps of Plant Diseases, No.October. Wallingford, UK: CABI, Map 1149 (Edition 1). [Online] [Available on: https://www.cabidigitallibrary.org/doi/10.1079/DMPD/20133421485] [Accessed: 12 September 2024].

Hall H.K., Hummer K., Jamieson A.J. 2009. Raspberry breeding and genetics. Plant Breeding Reviews 32: 281–290. DOI: https://doi.org/10.1002/9780470593806.ch2

Isogai M., Nakamura T., Ishii K. 2012. First report of raspberry yellows disease caused by Raspberry bushy dwarf virus in Japan. Journal of General Plant Pathology 78 (5): 360–363. DOI: https://doi.org/10.1007/s10327-012-0392-8

Isogai M., Matsudaira T., Ito M., Yoshikawa N. 2019. The 1b gene of raspberry bushy dwarf virus is a virulence component that facilitates systemic virus infection in plants. Virology 526: 222–230 DOI: https://doi.org/10.1016/j.virol.2018.10.025

Jevremović D., Leposavić A., Paunović S.A. 2019. Genetic diversity of Raspberry leaf blotch emaravirus in red raspberries from Serbia. Spanish Journal of Agricultural Research 17 (1): 1004. DOI: https://doi.org/10.5424/sjar/2019171-13861

Jones A.T., Murant A.F., Jennings D.L., Wood G.A. 1982. Association of raspberry bushy dwarf virus with raspberry yellows disease: reaction of *Rubus* species and cultivars, and the inheritance of resistance. Annals Applied Biology 100: 135–147. DOI: https://doi.org/10.1111/j.1744-7348.1982.tb07200.x

Jones A.T., Mayo M.A., Murant A.F. 1996. Raspberry bushy dwarf idaeovirus. p. 283–301. In: "The plant viruses. The polyhedral virions and bipartite RNA genomes" (B.D. Harrison, A.F. Murant, eds.). Springer. Boston, MA, 364 pp. DOI: https://doi.org/10.1007/978-1-4899-1772-0_11

Kerimbek N., Kapytina A., Pozharskiy A. 2022. Development of primer sets for detection of Raspberry leaf blotch virus and Raspberry leaf mottle virus by multiplex RT-PCR. Eurasian Journal of Applied Biotechnology 1: 36. DOI: https://doi.org/10.11134/btp.1.2022.4

Kimura M.A. 1980. Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. DOI: https://doi.org/10.1007/BF01731581

Kokko H.I., Kivineva M., Kärenlampi S.O. 1996. Single-step immunocapture RT-PCR in the detection of Raspberry bushy

dwarf virus. Bio Techniques 20 (5): 842–846. DOI: https://doi.org/10.2144/96205st03

Kolchenko M., Kapytina A., Kerimbek N., Pozharskiy A., Nizamdinova G., Khusnitdinova M., Taskuzhina A., Gritsenko D. 2023. Genetic characterization of Raspberry bushy dwarf virus isolated from red raspberry in Kazakhstan. Viruses 15 (4): 975. DOI: https://doi.org/10.3390/v15040975

Koloniuk I., Fránová J., Pˇribylová J., Sarkisova T., Špak J., Tan J.L., Zemek R., ˇCmejla R., Rejlová M., Valentová L. 2023. Molecular characterization of a novel Enamovirus infecting raspberry. Viruses 15 (12): 2281. DOI: https://doi.org/10.3390/v15122281

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35 (6): 1547–1549. DOI: https://doi.org/10.1093/molbev/msy096

Lu Y., McGavin W., Schnettler E., Yan F., Chen J., MacFarlane S. 2015. Newly identified RNAs of Raspberry leaf blotch virus encoding a related group of proteins. Journal of General Virology 96 (11): 3432–3439. DOI: https://doi.org/10.1099/jgv.0.000277

Martin D.P., Murrell B., Golden M., Khoosal A., Muhire B. 2015. RDP4: Detection and analysis of recombination patterns in virus genomes. Virus Evolution 1 (1): vev003. DOI: https://doi.org/10.1093/ve/vev003

Martin R.R. 2013. Viruses and virus disease of *Rubus*. Plant Disease 97 (2): 168–182. DOI: https://doi.org/10.1094/PDIS-04-12-0362-FE

Mavrič I., Marn M.V., Koron D., Žežlina I. 2003. First report of Raspberry bushy dwarf virus on red raspberry and grapevine in Slovenia. Plant Disease 87 (9): 1148. DOI: https://doi.org/10.1094/PDIS.2003.87.9.1148D

Mavrič I., Pleško M., Marn V., Lazarova S., Peneva V., Širca S., Urek G. 2014. First detection of Raspberry leaf blotch virus in red raspberry in Bulgaria. Journal of Plant Pathology 96 (2): 431–439. DOI: https://doi.org/10.4454/JPP.V96I2 .013

McGavin W.J., MacFarlane S.A. 2010. Sequence similarities between Raspberry leaf mottle virus, Raspberry leaf spot virus and the Closterovirus Raspberry mottle virus. Annals of Applied Biology 153 (3): 443–448. DOI: https://doi.org/0.1111/j.1744-7348.2010.00401

McGavin W.J., Mitchell C., Cock P.J.A., Wright K.M., MacFarlane S.A. 2012. Raspberry leaf blotch virus, a putative new member of the genus Emaravirus, encodes a novel genomic RNA. Journal of General Virology 93 (2): 430–437. DOI: https://doi.org/10.1099/vir.0.037937-0

Moury B., Desbiez C. 2020. Host range evolution of potyviruses: A global phylogenetic analysis. Viruses 12 (1): 111. DOI: https://doi.org/10.3390/v12010111

Muhire B., Varsani A., Martin D. 2014. SDT: A Virus Classification Tool Based on Pairwise Sequence Alignment and Identity Calculation. PLOS One 9 (9): 108–277. DOI: https://doi.org/10.1371/journal.pone.010827

Podedworny G., Tartanus M., Solecka D., Szakie A., Malusà E. 2024. Assessment of applying an integrated pest management strategy to control the raspberry leaf and bud mite, *Phyllocoptes gracilis* (Nal.) and its effect on the raspberry leaf metabolites. Journal of Plant Protection Research 64 (1): 52–60. DOI: https://doi.org/10.24425/jppr.2024.149152

Pozhylov I., Snihur H., Budzanivska I. 2021. Phylogenetic analysis of Ukrainian isolate of raspberry leaf blotch virus. AGROFOR 6 (1): 19–25. DOI: https://doi.org/10.7251/AGRENG2101019P

Pupola N., Lepse L., Kale A., Morocko-Bicevska I. 2009. Occurrence of RBDV in Latvia and virus elimination in vitro by chemotherapy. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture. Sodininkystë ir Darþininkystë 28 (3): 165–172. [Online] [Available on: https://www.cabidigitallibrary.org/doi/pdf/10.5555/20103215960] [Accessed 13 January 2025].

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. DOI: https://doi.org/10.1094/PDIS-12-12-1198-PDN

Ukrainian Ministry of Agriculture. 2023. Register of certificates for seeds and/or planting material. [Online] [Available on: https://minagro.gov.ua/file-storage/reyestr-sertifikativ-na-nasinnya-taabo-sadivnij-material] [Accessed 13 January 2025].

Riaba I., Pavliuk L., Udovychenko K., Tryapitsyna N. 2019. Virusfree clones selection in the raspberry (*Rubus idaeus* L.) and blackberry (*Rubus caesis* L.) mother gardens. Sadivnyctvo 74: 153–160. DOI: https://doi.org/10.35205/0558-1125-2019-74-153-160 [In Ukrainian]

Saitou N., Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4 (4): 406–425. DOI: https://doi.org/10.1093/oxfordjournals.molbev.a040454

Schoch C.L., Ciufo S., Domrachev M., Hotton C.L., Kannan S., Khovanskaya R., Leipe D., Mcveigh R., O'Neill K., Robbertse B., Sharma S., Soussov V., Sullivan J.P., Sun L., Turner S., Karsch-Mizrachi I. 2020. NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford). baaa062. PubMed: 32761142 PMC: PMC7408187. [Available on: https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=2732890] [Accessed: 23 August 2024]

Sproge L., Strautina S. 2020. Influence of Raspberry bushy dwarf virus on pollination of red raspberry (*Rubus idaeus* L.) cultivars. Agronomy Research 18 (4): 2776–2784. DOI: https://doi.org/10.15159/AR.20.227

Stephens M.J., Buck E.J., Tahir J. 2016. Mapping a potential resistance gene for Raspberry bushy dwarf virus in red raspberry. Acta Horticulturae 1133 (18): 121–128. DOI: https://doi.org/10.17660/ActaHortic.2016.1133.18

Susi H., Rajamäki M.L., Artola K., Jayaraj-Mallika F.R., Valkonen J.P.T. 2018. Molecular detection and characterisation of black raspberry necrosis virus and Raspberry bushy dwarf virus isolates in wild raspberries. Annals of Applied Biology 173 (2): 97–111. DOI: https://doi.org/10.1111/aab.12438

Tryapitsyna N.V., Yareschenko O.M., Medvedeva T.V., Udovychenko K.M., Lushpigan O.P., Suprun K.I. 2013. Viruses dominating in plantations of bush berry crops. Sadivnyctvo 67: 193–203. [Online] [Available on: http://nbuv.gov.ua/UJRN/sadiv_2013_67_30.] [Accessed 11 January 2025] [In Ukrainian]

UKRSTAT. 2022. Areas, gross harvests and productivity of agricultural crops by their types by regions. [Online] Available on: https://www.ukrstat.gov.ua/operativ/operativ2022/sg/pvzu/archpvxureg.htm] [Accessed: 19 August 2024].

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. DOI: https://doi.org/10.1007/s00705-010-0912-9

Ward J.A., Boone W.E., Moore P.P., Weber C.A. 2012. Developing molecular markers for marker assisted selection for resistance to Raspberry bushy dwarf virus (RBDV) in red raspberry. Acta Horticulturae 946 (6): 61–66. DOI: https://doi.org/10.17660/actahortic.2012.946.6

Yu C., Karlin D.G., Lu Y., Macfarlane S. 2013. Experimental and bioinformatic evidence that RLBV P4 is a movement protein of the 30K superfamily. Journal of General Virology 94 (9): 2117–2128. DOI: https://doi.org/10.1099/vir.0.053256-0

Zindović J., Viršček Marn M., Mavrič I., Pleško M. 2015. First report of Raspberry leaf blotch virus (HTS/RT-PCR) in red raspberry in Montenegro. Journal of Plant Pathology 97 (2): 398. DOI: https://doi.org/10.4454/JPP.V97I2.024