Comparison of *Raspberry bushy dwarf virus* isolates from Hungary and Slovenia

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Abstract

In 2006 and 2007 samples of grapevine and *Rubus* species were collected and analysed by DAS-ELISA to survey the presence of *Raspberry bushy dwarf virus* (RBDV) in Slovenia and Hungary. Seven varieties of raspberry from one Hungarian collection orchard were found to be infected. In Slovenia the presence of RBDV was confirmed only in three samples of wild *Rubus*. None of the 133 samples from different locations in Hungary proved to be infected with RBDV, although this virus is found to be widely distributed in grapevine in neighbouring Slovenia. Serological characterisation with three monoclonal antibodies (R2, R5 and D1) was performed on positive samples. Selected positive samples were partially sequenced. The results of serological and molecular analyses were compared with the analyses of raspberry and grapevine isolates obtained in Slovenia from other projects and published RBDV sequences from the GeneBank database to study the variability among hosts and locations. Isolates from grapevine grouped separately from the black raspberry isolate and all the red raspberry isolates. RBDV isolates from Hungarian samples formed a subgroup within red and black raspberry group.

Keywords: RBDV, variability, *Rubus*, raspberry, grapevine, sequences, monoclonal antibodies

Introduction

Raspberry bushy dwarf virus is found to infect *Rubus* species worldwide and can cause serious damage in certain varieties. In 2003 this virus was reported to infect grapevine, too. This was the first report of RBDV naturally infecting a host outside the genus *Rubus* (Mavrič et al. 2003). Later RBDV was found to be widespread in Slovenia on numerous white and red grapevine varieties (Virščck Marn and Mavrič 2006, Mavrič Pleško et al. 2009). Within the framework of a bilateral project between the Republic of Hungary and the Republic of Slovenia entitled “Study of *Raspberry bushy dwarf virus* (RBDV) infection in grapevine and *Rubus* plantations”, grapevine and wild and cultivated *Rubus* samples were collected in Hungary and Slovenia to survey the presence of this virus on host plants in both countries and to study serological and genetic differences among hosts and locations. Since until then RBDV had only been found in Slovenia and on a limited number of grafts in Serbia (Paunović, personal communication), the survey of grapevine in Hungary was of special interest.

Material and methods

In Hungary 52 leaf samples of grapevine (17 different varieties) were collected in five different vineyards in surrounding areas of Nagyréde and Kecskemét in May 2007. At the same time 49 leaf samples of raspberry (varieties ‘Autumn Bliss', 'Blissy' - a Hungaran selection of 'Autumn Bliss', 'Chilliwack', 'Comox', ‘Fertödi Kétszertermő', 'Fertődi Vénusz', 'Fertődi Zamatos', 'Glen Ample', 'Glen Moy', 'Golden Bliss', 'Malling Exploit', 'Nootka', ‘Summit', 'Tulameen', three samples of unknown raspberry variety, one *Rubus* hybrid and one wild *Rubus* plant) were taken at seven locations around Nagyréde and Pölöske. In November 2008, 81 wood samples of grapevine were collected from 19 grapevine varieties typically grown in Hungarian vine growing regions (Transdanubian North Balaton – Badacsony; North Hungary Bükk Mountains - Eger; North Hungary Hegyalja - Tokaj). In Slovenia 18 samples of eight raspberry varieties (‘Autumn Bliss', 'Glen Ample', 'Himbotop', 'Loch Ness', 'Meeker', 'Polka', 'Tulameen', and 'Willamette') were taken from orchards of the Posavje region in October 2007. Wild *Rubus* was collected in the Gorenjska and the Notranjska region in September 2008. Altogether 43 samples were taken from 14 locations.

Collected samples were tested for the presence of RBDV by DAS-ELISA (Loewe Biochemica or Bioreba AG) according to manufacturer’s instructions, except that only 100 µl per well was used. Absorbance was read at 405 nm in a Sunrise Remote Control Reader (TECAN Austria GmbH). Samples were considered positive when the mean absorbance value of a sample after three hours exceeded the threshold. The threshold was set as at least two times the mean absorbance value of healthy controls. All of the positive samples from Hungary and some samples of RBDV infected grapevine, red raspberry and wild *Rubus* from Slovenia (collected in the frame of other projects) were tested

with three monoclonal antibodies R2, R5 and D1 (R. R. Martin, ADA-ARS, Corvallis OR, USA) to differentiate isolates in TAS-ELISA using the protocol described by Mavrič Pleško et al. (2009). Samples of raspberries from Slovenia and the wild *Rubus* sample were collected in 2005 and were kept as freeze dried material at -20°C. These samples were diluted with DAS-ELISA extraction buffer in a 1:200 ratio.

IC RT-PCR with primer pairs CPUP and RNA12 for the coat protein and MPUP and MPLO for the movement protein (Mavrič Pleško et al. 2009) were performed for three positive raspberry samples from Hungary. The amplicons were purified and cloned into pGEM-T easy vector (Promega) according to the manufacturer's instructions. Transformed colonies were selected by blue/white screening and subsequent PCR. Plasmids were isolated from selected colonies and sent for sequencing (Macrogen, Korea). Analysed sequences (BioEdit version 7.0.5.3, Hall 1999) and their deduced amino acid sequences were compared with sequences of RBDV published in the GeneBank database (http://www.ncbi.nlm.nih.gov/). Phylogenetic analyses were conducted using MEGA 4 programme (Tamura et al. 2007). Phylogenetic trees were constructed using neighbour-joining method and bootstrap analysis with 1000 replications.

Results

None of the 133 samples of numerous grapevine varieties taken in several Hungarian grapevine growing regions proved to be positive for RBDV. Seven raspberry varieties from a collection in Pölöske ('Autumn Bliss', 'Blissy', 'Comox', 'Glen Moy', 'Golden Bliss', 'Summit' and 'Tulameen') were found to be infected with RBDV. In Slovenia the presence of RBDV was confirmed only in three out of 43 samples of wild *Rubus* collected from woods. All of the tested RBDV positive raspberry samples with the exception of one freeze dried sample of variety 'Willamette' tested positive for all three isolate groups. Variety 'Blissy' from Hungary and variety 'Fall Gold' from Slovenia showed a quick reaction with all three monoclonal antibodies whereas the rest of raspberry samples reacted slowly with D1. Grapevine isolates and the freeze dried sample of raspberry variety 'Willamette' reacted only with R2 and R5 (Fig. 1).

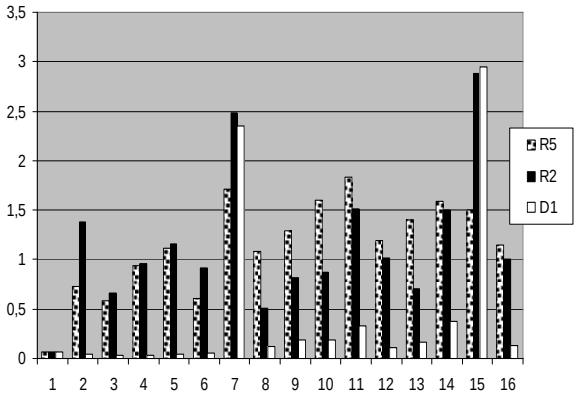

Fig. 1 Results of TAS-ELISA (absorbance was read at 405 nm after 3 hours) for monoclonal antibodies R5, R2 and D1 from October 2007: 1 = negative control x 2; 2 = grapevine variety 'Chardonnay' (Slovenia, 2007); 3 = grapevine variety 'Zweigeld' (Slovenia, 2007); 4 = grapevine variety 'White Riesling' (Slovenia, 2007); 5 = grapevine variety 'Furmint' (Slovenia, 2007); 6 = red raspberry variety "Willamette' (Slovenia, 2005, freeze dried); **7 =** red raspberry variety 'Fall Gold' (Slovenia, 2005, freeze dried); 8 = wild *Rubus* (Slovenia, 2005, freeze dried); 9 = red raspberry variety 'Meeker' (Slovenia, 2005, freeze dried); 10 = red raspberry variety 'Autumn Bliss' (Hungary, 2007); 11 = red raspberry variety 'Summit' (Hungary, 2007); 12 = red raspberry variety 'Comox' (Hungary, 2007); 13 = red raspberry variety 'Golden Bliss' (Hungary, 2007); 14 = red raspberry variety 'Tulameen' (Hungary, 2007); 15 = red raspberry variety 'Blissy' (Hungary, 2007); 16 = red raspberry variety 'Glen Moy' (Hungary, 2007).

Phylogenetic analyses performed with nucleotide sequences (not shown) and deduced amino acid sequences of coat protein (Fig. 2) and movement protein (Fig. 3) showed three major groups of isolates. *R. multibracteatus* grouped separately and was genetically most distant from other isolates. Isolates from grapevine formed a separate group. The black raspberry isolate and all the red raspberry isolates formed the third group, within which Hungarian ones clustered together in a subgroup**.**

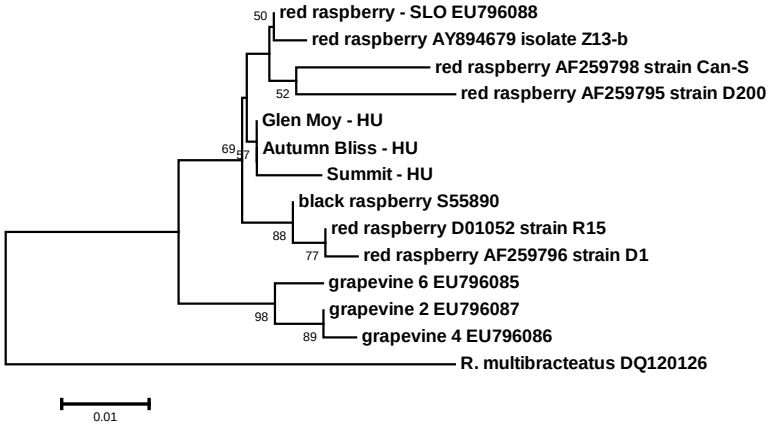

Fig. 2 Polygenetic tree based on the coat protein amino acid sequences

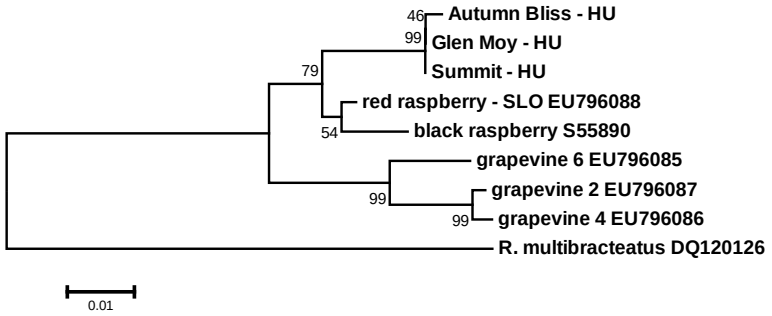

Fig. 3 Polygenetic tree based on the movement protein amino acid sequences

Discussion

RBDV infection was not found in grapevine in Hungary. This is rather surprising considering the abundant presence of this virus in Slovenian vineyards, especially in the NE Slovenia towards the Hungarian border (Viršček Marn and Mavrič, 2006; Mavrič Pleško et al. 2009), and the fact than many of the sampled varieties are grown both in Hungary and Slovenia. The exchange of plant material between the two countries is not very frequent, which could explain the absence of RBDV infection in Hungary. In continuation of our work we will therefore survey Hungarian vineyards close to the border with Slovenia where plant material exchange is probably more common.

In *Rubus* RBDV is distributed naturally by pollen to progeny and pollinated plant. In grapevine all the 390 tested seedlings grown from seeds collected on the RBDV infected grapevine were free from RBDV, which indicates that the virus is not transmitted or transmitted at low efficiency in grapevine (Mavrič Pleško et al. 2009). RBDV was detected by nested RT-PCR in *Longidorus juvenilis* nematodes from the soil in the vineyard infected with this virus. Since viruses can be detected in nematodes also if these are not their vectors, the possible role of *L. juvenilis* in RBDV transmission is still under investigation at the Agricultural Institute of Slovenia (Mavrič Pleško et al. 2009). The spread of viruses by nematodes is rather limited, so RBDV was probably distributed within Slovenia with grapevine propagation material, either rootstocks, scion material or both, but its origin is still unknown.

All the grapevine varieties reacted with monoclonal antibodies R5 and R2 but not with D1 (Fig. 1). A sample of the raspberry variety ‘Willamette’, collected in 2005 and stored as a freeze dried material, showed the same reaction as grapevine, but the results of testing with D1 monoclonal antibodies were closer to the threshold as in grapevine and the

long storage might have deteriorated the material. The analysis will have to be repeated to confirm or reject the finding that red raspberries can react the same way as grapevine with the three monoclonal antibodies. Two of the studied red raspberry samples showed high absorbance values (at 405 nm) for all three monoclonal antibodies whereas the others had high values for R5 and R2, but reacted slowly with D1. Isolates with different reactions to D1 were found in the same collection orchard, so two or more isolates must have been introduced in the collection with planting material of different varieties. There are also small genetic differences among the tree sequenced Hungarian varieties, which could be the consequence of a different origin of infected planted material or/and due to mutations occurring in the field. Nevertheless Hungarian isolates proved to be very similar to each other and formed a subgroup within the group of red and black raspberry isolates. Grapevine isolates formed a separate group. *R. multibracteatus* isolate proved to be genetically more distant. The infection of this plant species was reported by Chamberlain et al. in 2003 on a plant from China. The *R. multibracteatus* isolate reacted only with monoclonal antibodies R5 and R2 and not with D1 (Chamberlain et al. 2003) and was thus serologically similar to grapevine isolates. Serological and genetic data about RBDV are still scarce and more data is needed to understand the variability and epidemiology of this virus. In the laboratory of the Agricultural Institute of Slovenia work to obtain more sequences of raspberry, wild *Rubus* and grapevine isolates is in progress.

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