Detection of *Blueberry red ringspot virus* in highbush blueberry cv. 'Coville' in Slovenia

Mavrič Pleško, I., Viršček Marn, M., Koron, D.
Agricultural Institute of Slovenia, Hacquetova 17, SI-1000 Ljubljana, Slovenia. Email: irena.mavric@kis.si

Abstract

Blueberry red ringspot virus (BRRSV) infects blueberries and is present in USA. It causes red ringspots or red blotches on one year old stems or older. In mid- to late summer reddish-brown spots develop on older leaves. In some blueberry cultivars also fruit symptoms, circular areas of light colour and/or fruit deformations, can be seen. In spring 2008 BRRSV was detected in symptomatic bark from blueberry cv. 'Coville' showing typical BRRSV symptoms. The obtained PCR product was sequenced and the identity of the virus confirmed. To our knowledge this was the first finding of BRRSV in Slovenia.

Keywords: BRRSV, Vaccinium, PCR, detection

Introduction

Blueberry red ringspot virus (BRRSV) is a member of genus *Soymovirus* in the family Caulimoviridae. It is known to infect *Vaccinium corymbosum*, *V. formosum*, *V. australe* and probably *V. macrocarpon*. Many blueberry cultivars are sensitive to BRRSV, like 'Blueray', 'Bluetta', 'Coville', 'Earlyblue' and others. Cv. 'Bluecrop' is reported to be field-resistant and cv. 'Jersey' is field-immune. BRRSV causes red ringspots or red blotches on older stems. In mid- to late summer reddish-brown spots develop on older leaves. The spots are prominent on the upper surface of the leaf. Similar symptoms can be caused by powdery mildew (*Microsphaera alni* var. *Vaccinii*) except that leaf spots are prominent on both sides of the leaf. In some cultivars fruit symptoms, circular areas of light colour and/or fruit deformations can be seen. The disease can significantly reduce yield.

The disease is present in USA. Paulechova (1972) has reported the occurrence of red ringspot in former Czechoslovakia on wild *Vaccinium myrtillus*. She did not detect the virus but only showed that powdery mildew was not present. The identity of BRRSV in the Czech Republic was confirmed in 2009. In 2009 BRRSV was reported also in Japan (Isogai et al., 2009).

On one plant of highbush blueberry in an introduction plantation at Brdo pri Lukovici, symptoms indicating BRRSV infection were observed. Red rings appeared on some of the stems and also red rings or spots were observed on some leaves. At the harvest time spots of a light colour were observed in ripening fruits. The aim of our work was to introduce PCR for virus identification into our laboratory and to confirm BRRSV infection of symptomatic blueberry.

Material and methods

In spring 2008 young non-symptomatic leaves and bark from symptomatic blueberry cv. 'Coville' were sampled for virus identification. DNA was isolated from two samples of young leaves and one sample of symptomatic bark tissue using DNeasy Mini Kit (Qiagen) according to manufacturer's instructions. The quality and quantity of isolated DNA was checked on an agarose gel. Serial 10-fold dilutions in water were prepared from isolated DNA and plant DNA control PCR using primers Gd1 and Berg54 was performed (Ward, 2007). Primers RR13 and RR14 (Glasheen et al., 2002) were used in subsequent PCR assays on diluted DNA to detect the virus. Amplified products were analysed on 1% agarose gels and stained with ethidium bromide. An amplification product of the expected size, approximately 490 bp, was obtained and subsequently sequenced (Macrogen, Korea) to confirm it represented sequence of BRRSV.

Results and discussion

For undiluted DNA only one sample (symptomatic bark) produced the PCR product of expected size. However with a dilution 1/10 two samples yielded a product, and with a dilution of 1/100 and 1/1000 all three samples produced the amplification product of expected size. This indicates the presence of PCR inhibitors in isolated DNA. In subsequent BRRSV specific PCR assays we used the same dilutions and quantities of isolated DNA as in the plant DNA control PCR. Only DNA isolated from symptomatic bark produced a PCR product. With undiluted DNA and dilutions of 1/10 and 1/100 we obtained the PCR product of expected size. With a dilution of 1/1000 no PCR product was observed.

A BRRSV specific amplification product of approximately 490 bp was sequenced and the infection of blueberry plant with BRRSV confirmed. To our knowledge this was a first finding of BRRSV in Slovenia.

BRRSV is known to be present in blueberries in the USA and can cause symptoms on some of the cultivars. It was first described in New Jersey in 1950 and is still a problem there. In recent years it has been reported in Arkansas, Michigan, Connecticut, Massachusetts, New York, North Carolina and Oregon (Martin et al., 2009).

In Slovenia, the symptoms of BRRSV were observed on one plant in a plantation over 20 years old. No symptoms were observed on other plants in the same plantation. No virus was detected in plants growing adjacent to the infected plant. This result indicates that the virus is not spreading in our conditions, which is similar to observations in Michigan and Oregon, where the virus does not appear to spread (Martin et al., 2009).

Using our method, the virus could be detected only in symptomatic tissue and not in young leaves, which are recommended for sampling and testing in New Zealand (Ward, 2007). In future work we plan to determine the reliability of BRRSV detection throughout the growing season in different symptomatic and asymptomatic plant tissues to optimize the ability to detect this virus and provide more accurate information for growers and extension services.

Literature

Gillett, J. M.; Ramsdell, D. C.; 1988: Blueberry red ringspot virus. DPV No. 327. Association of Applied Biologists.

Glasheen, B. M.; Polashock, J. J.; Lawrence, D. M.; Gillett, J. M.; Ramsdell, D. C.; Vorsa, N.; Hillman, B.I.; 2002: Cloning, sequencing, and promoter identification of Blueberry red ringspot virus, a member of the family Caulimoviridae with similarities to the 'Soybean chlorotic mottle-like' genus. Archives of Virology **147**, 2169-2186. DOI 10.1007/s00705-002-0866-7.

Isogai, M.; Ishii, K.; Umemoto, S.; Watanabe, M.; Yoshikawa, N.; 2009: First report of blueberry red ringspot disease caused by Blueberry red ringspot virus in Japan. Journal of General Plant Pathology **75**, 140-143.

Martin, R. R.; Tzanetakis, I. E.; Caruso, F. L.; Polashock, J. J.; 2009: Emerging and reemerging virus diseases of blueberry and cranberry. Acta Horticulturae **810**, 299-304.

Ramsdell, D. C.; Kim, K. S.; Fulton, J. P.; 1987: Red ringspot of blueberry. In: Virus diseases of small fruits, USDA Agriculture handbook No. **631**, 121-123.

Ward, L.; 2007: *Vaccinium* Post-Entry Quarantine Testing Manual - 5 March 2007. Biosecurity New Zealand.