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Review Article

Current Status of Virus Diseases in Highbush Blueberries in Turkey

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Abstract

The Black Sea Region of Turkey, northeastern part of Anatolia, is one of the main germplasm centers of several Vaccinium and Ericaceous plant species. Caucasian whortleberry (*Vaccinium arctostaphylos*), bilberry (*Vaccinium myrtillus*) and lingonberry (*Vaccinium vitis-idea*) have been naturally grown in the forests and plateaus over the centuries in Turkey. However commercial blueberry production by using northern high bush blueberries (*Vaccinium corymbosum*) started in the 2000s in the country. To date there has been very limited data on the presence of blueberry viruses in Turkey. Recently, the first large-scale survey of viruses in highbush blueberry plantations in the country was carried out in 2014-2015. The aim of this study was testing blueberry plants for some important viruses in *Vaccinium* species such as *Blueberry leaf mottle* virus (BLMoV), *Blueberry scorch virus* (BlScV), *Blueberry shock virus* (BlShV) and *Blueberry shoestring virus* (BSSV) by ELISA and *Blueberry mosaic associated virus* (BlMaV) by RT-PCR analysis. Blueberry samples were collected from Samsun, Giresun, Rize, Trabzon and Artvin provinces located in the Black Sea Region in 2014 and 2015. The most common symptoms were observed as leaf crinkling, reddening, ringspots and mosaics on the blueberry leaves. DAS-ELISA tests showed that none of the 135 analyzed plant samples were positive for the presence of BLMoV, BlScV, BlShV, BSSV, TRSV and ToRSV. Three samples out of ten symptomatic blueberry cvs. Jersey, Bluejay and Ivanhoe collected from Rize province was found positive for BlMaV by RT-PCR and 350 bp fragment was succesfully amplified. Further studies on molecular detection and characterization of Turkish BlMaV isolates are still under investigation.

Common and New Emerging Viruses of Blueberries

Blueberries belong to the genus *Vaccinium* in the family *Ericaceae*. The family has more than 3,500 species growing in all latitudes, from the tropics to the polar regions. A common feature among members of the family is that they grow in acidic, many times, nutrient-deprived soils [1]. Their popularity has been increasing recently as new research discloses their nutritional benefits. Highbush blueberries have been affected by virus-related diseases that have cause multimillion dollar losses [2,3]. A few species of viruses were isolated from the bushes of blueberry such as *Blueberry scorch virus* (BlScV), *Blueberry shock virus* (BlShV), *Blueberry shoestring virus* (BSSV), *Blueberry leaf mottle virus* (BLMoV), *Blueberry red ringspot virus* (BRRSV), *Tobacco ringspot virus* (TRSV), *Tomato ringspot virus* (TomRSV) and *Peach rosette mosaic virus* (PRMV). Some of those pathogens are polyphages, for which the highbush blueberry is one of the many host plants. They are: TRSV, ToRSV and PRMV. For BlShV, BSSV or BRRSV, that plant is the only so far recognized host [3,4]. There are three main means of transmission for viruses that infect *Vaccinium* spp. , by nematodes, aphids and pollen [5]. Most of the work on the viruses of blueberries has been done in Europe and North America. As a result there is little information on the viruses that occur in *Vaccinium* spp. in other parts of the world.

Recently some more additional viruses were reported in blueberries. A recent example of this is the occurrence of *Blueberry necrotic ring blotch virus* (BNRBV) in the southeastern U.S., which occurred only a few years after the industry there began to expand rapidly [5]. Based on genome organization and sequence divergence, BNRBV represents a new genus of plant viruses called *Cilevirus* [6]. In order to detect BLMoV, BSSV, BlScV, BlShV, BRRSV, TomRSV and PRMV in the blueberry bushes, the serological test ELISA could be applied. Although ELISA reagents have been developed to detect BRRSV in symptomatic leaves but not in symptomless leaves from an infected bush, the BRRSV ELISA test has not been robust and is not currently recommended [7]. On the other hand, BlMaV and BNRBV can be only detected with the help of the PCR technique [6,8,9]. There are no chemical means to control plant viruses directly, so once field plants become infected the only way to get rid of the virus is to remove the infected plants. First, the virus involved must be identified and then the appropriate control measures can be developed.

Overview of Blueberry Viruses in Turkey

To date there has been very limited data on the presence of blueberry viruses in Turkey. Recently, the first large-scale survey of viruses in highbush blueberry plantations in the country was carried out in 2014-2015 [10]. The aim of this study was the detection and identification of some important viruses in the highbush blueberry on plantations located in the Blacksea region of Turkey using the serological DAS-ELISA test and the RT-PCR technique. The collected blueberry samples were tested for Blueberry leaf mottle virus (BLMoV), Blueberry scorch virus (BlScV), Blueberry shock virus (BlShV) and Blueberry shoestring virus (BSSV) by ELISA and Blueberry mosaic associated virus (BlMaV) by RT-PCR. In this study, the investigation material comprised 135 highbush blueberry plants grown in Blacksea region of Turkey. Leaf samples of blueberry plant which were showing virus-like symptoms were collected during spring 2014 and 2015. DAS-ELISA reagents for with specific antibodies for BLMoV, BSSV, BlScV, BlShV were supplied from Agdia Incorporated (USA) whereas TRSV and ToRSV were supplied by Bioreba-Switzerland. The most common symptoms were observed as leaf crinkling, reddening, ringspots and mosaics on the leaves in 8- to 20-year-old mature highbush blueberry cvs Jersey, Bluejay, Ivanhoe, Patriot and Bluecrop in various plantings in Blacksea region of Turkey. Symptoms in cvs Jersey, Bluejay, Ivanhoe consisted of reduced vigor and mosaics on the leaves (Figure 1), distorted and crinkled apical leaves with dark reddish lesions (Figure 2).

Figure 1: Mosaic symptoms on blueberry leaves. From yellow to light green mosaic pattern induced by *Blueberry mosaic associated virus*.

Figure 2: Leaf crinkling and reddening on the leaves due to unknown factors.

Preliminary DAS-ELISA tests on the presence of BLMoV, BlScV, BlShV, BSSV, TRSV and ToRSV revealed that none of the 135 analyzed leaf samples were positive. Some of the symptomatic samples that were resembling virus-like symptoms such as leaf crinkling and reddening on the leaves and sometimes on the fruits (Figure 2) were found negative for all tested viruses. Symptoms typical for blueberry mosaic disease were observed only on the leaves of 3 plants in 1 location. The plants, cvs. Jersey, Bluejay and Ivanhoe showed yellow, or yellowish green mosaic pattern and vein clearing. Leaf mottling and mosaic symptoms suggesting the presence of BlMaV were observed in a commercial blueberry orchard in Rize province located in Blacksea region of Turkey. RT-PCR analyses was performed to confirm the presence of BlMaV. A product of expected size was obtained with three symptomatic samples from this orchard and obtained sequences were all identical. Further molecular characterization of BlMaV isolates is still in progress.

Conclusion

The genus *Vaccinium* (family *Ericaceae*) consists of about 450 species and grow wild around the world and there are many names given to the numerous varieties that produce edible fruits, such as blueberry, cranberry, bilberry and others. Blueberries and cranberries are the only *Vaccinium* species commercially grown and Blacksea Region of Turkey is one of the main origins of Caucasian whortleberry (*Vaccinium arctostaphylos*), bilberry (*V. myrtillus*), lingonberry (*V. vitis-idea*) and bog blueberry (*V. uliginosum*) [11,12]. Highbush blueberries have been affected by virus-related diseases that have cause important economical losses on crop. The cost of virus diseases is massive in perennial fruit crops such as blueberry and cranberry that require a large initial investment to prepare, plant, and establish fields to the point of maturity and full productivity [5]. Most of the analyzed samples in this study were negative on the presence of any of the analyzed viruses except BlMaV which was the most frequent virus detected in this study. The presence of BlMaV was confirmed in three samples out of ten symptomatic samples. Blueberry mosaic disease (BMD) has been known for more than 60 years, but the causative agent was described only recently [9]. This new virus is belonging to the *Ophiovirus* genus (Martin et al. 2012) and tentatively named as *Blueberry mosaic associated virus* (BlMaV) (Thekke-Veetil et al. 2014). It has been described in many countries, but so far the presence of BlMaV has been confirmed in the USA, Slovenia, Serbia and Japan [9,13-16]. The presence of BlMaV was also recently reported in Turkey [17]. The lack of health control of the imported plant material and the easiness of transmission during vegetative propagation resulted in the fact that the presence of viruses on the local plantations became a fact. On the other hand a bigger number of plants infected by viruses in some countries may also result from different conditions of the development of the population of vectors. As ophioviruses are transmitted by soil-borne fungi, BlMaV could survive in the spores of its vector(s) in infected roots and soil leading to disease spread [14]. The discovery of BlMaV in Turkey will allow for the development of sensitive detection assays that will assist in nursery and field screening and developing control strategies for the virus.

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