Cereal viruses – brome mosaic virus: prevalence and diagnostics

D D Zvyaginceva¹, O O Beloshapkina¹, A A Lopatkin², Y A Shneider² and O N Morozova²

¹ - Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, Moscow, Russia
² – All-Russian Science-Research Institute of Plant Quarantine, Bykovo, Moscow region, Russia

Abstract. Analysis of the prevalence and bioecological characteristics of the brome mosaic virus (BMV), which is a widespread polyphage virus that causes significant economic damage to cereals. Experimental studies were carried out in the laboratory of the All-Russian Science-Research Institute of plant Quarantine (Moscow region), visual examinations of crops and sampling of winter wheat in the tillering phase were carried out in the farms of the Rostov region, the Chechen Republic, in the Moscow region (Russian State Agrarian University - Moscow Timiryazev Agricultural Academy) in 2019-2020. Route surveys of fields in each region were carried out in the spring. The visually estimated prevalence of viroses on average did not exceed 0.5%. The detection and identification of the brome mosaic virus in plants with symptoms of mosaic and mottling was carried out by the method of classical PCR with visualization of the results by gel electrophoresis. Brome mosaic virus was detected by RT-PCR in winter wheat samples from the Rostov region. No brome mosaic virus was detected in winter wheat plants from the Chechen Republic, Moscow region, although the leaves showed virus-like symptoms.

Key words: wheat, brome mosaic virus, prevalence, identification, PCR.

1. Introduction

In recent decades, the prevalence and economic importance of viruses that infect cereals have increased significantly in the world. Some of them have become serious threats to the cultivation of essential crops such as wheat and barley. At the same time, the number of cereal viruses detected in the world is growing and is currently about 90 species belonging to different families and genera. This growth is due to the impact of stress anthropogenic factors and techniques used in intensive technologies. A characteristic feature of the species composition is the predominance of polygostal pathogens, polyphagous viruses with a wide range of susceptible plant species. These viruses include the Brome mosaic virus (BMV), which infects about 160 plants from seven different families, including 50 types of cereals - barley, wheat, rye, triticale, oats, corn and others [1]. In addition to cereals, BMV has been found in soy [2], beets, cucumber, and beans [3]. The hosts of this virus can also be wild plants of the Poacea family, such as Bromus inermis, Dactylis glomerata, Festuca pratensis, Festuca arundinacea, Elytrigia repens, Phleum pretense and Poa pratensis [4]. Thus, cultural phytocenoses, as well as wild plants, are reserves of the brome mosaic virus.
One of the significant factors in changing the species composition and expanding the range of virus host plants are vectors. The brome mosaic virus is spread in agroecosystems in a non-persistent manner by beetles of the genus *Diabrotica*, *Oulema melanopus* and *Chaetocnema aridula* [5]. In Europe cases of transmission by beetles (*Phyllotreta vittula*), aphids (*Diuraphis noxia*) and nematodes (*Longidorus* sp.) was reported. There are reports of the transfer of BMV by nematodes of the genera *Longidorus* and *Xiphinema* under laboratory conditions, however, no relationship between the occurrence of these nematodes and the fire mosaic virus in nature has been found [7]. The virus is easily transmitted mechanically, with the sap of infected plants. BMV virions are spherical, 25-30 nm in diameter. They contain three RNA molecules of 3.2, 2.9 and 2.1 kb in length, packed into separate viral particles.

This virus was first described in the USA in 1942, and in Russia it was detected in 1964 [1]. To date, there is information about the spread of BMV in a number of European countries (Belarus, Ukraine, Moldova, Poland, Lithuania, Estonia, Sweden, Germany, France, Turkey), in the USA, Canada, South Africa and Australia. The study of this virus is associated not only with its widespread distribution, but also with the harmfulness to grain crops. In general, yield losses amount to 61%, while the quality of grain decreases [8].

It is difficult to diagnose viral diseases visually, since their external signs are similar to the symptoms of other diseases, non-infectious or infectious. Infected plants exhibit mottling, chloroticity, mosaicism, necrotization and deformation of leaves, and growth inhibition [9]. However, this method is widely used to assess the mass sowing of grain crops, which is of practical importance. A group of promising varieties of various origins from the collection of VIR named after V.I. N.I. Vavilov (in the Orenburg region) without signs of infection by viral diseases for use in breeding [10, 11].

Since visual assessment of symptoms is not a reliable method for diagnosing virosis, serological (ELISA) and molecular genetic methods are being developed and tested. To diagnose the brome mosaic virus, the method of indicator plants can be used, which are used as corn seedlings. On seedlings of many maize varieties, chlorotic streak develops along the leaf veins with further necrotization and leaf death, which are typical symptoms of BMV. The brome mosaic virus is the only species of the genus *Bromovirus* (family *Bromoviridae*) that infects plants of the Poacea family, making ELISA an effective method for diagnosing this virus in plants of this family. On plants of the *Fabaceae* family, the use of ELISA for the diagnosis of BMV is problematic. This method can show false positive results due to the presence of other bromoviruses serologically close to the brome mosaic virus [12]. Polymerase chain reaction (PCR) is a more reliable method for detecting and identifying brome mosaic virus.

The aim of our work is to adapt the technique for diagnosing the brome mosaic virus by RT-PCR and test it on wheat samples from different regions.

### 2. Conditions, materials and methods

Laboratory studies were carried out at the All-Russian Science-Research Institute of plant Quarantine, visual examination of crops and sampling of winter wheat in the tillering phase were carried out in the farms of the Rostov region (20 hectares), the Chechen Republic (Starosunzhensky district) (27 hectares), as well as in the fields of the Russian State Agrarian University - Moscow Timiryazev Agricultural Academy (2 ha) in 2019-2020. Route surveys of fields in each region were carried out in the spring, visually assessing the prevalence of viroses at 10-20 points for 10 plants located at random.

Plants (11-15 pcs.) with virus-like symptoms such as chloroticity and mosaicism of leaves were selected for the PCR study. Object - brome mosaic virus (BMV), genus *Bromovirus*, family Bromoviridae in fresh leaf samples.

Total RNA from leaf fragments was isolated using the "Proba NK" kit from "Agrodiagnostika", according to the manufacturer's instructions. RT-PCR (reverse transcription - polymerase chain reaction) was performed in one step using a PCR-mix kit from Syntol. The composition of the reaction mixture: 10 μl of a 2.5-fold reaction mixture, 0.75 MgCl2 (25 mM), 0.4 μl of MMLV reverse transcriptase (50 U / μl), 1 μl of forward and reverse primers, 1 μl of RNA and water to 25 μl.

We used the following primers [13]:

BMVep-F GATCTATGTCTCCTAATTCCG

BMVep-R GATTATAGTGCATCATATTCCG
BMVcp-R CTAGTCAGGGGCTCTCCGAGC

They were previously tested on BMV PV-0194 DSMZ isolate. These primers are complementary to a region of the RNA-3 molecule. Product size - 626 bp. Amplification was carried out in the following mode: 45°C for 15 minutes, 94°C for 5 minutes, then 35 cycles, including denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, and elongation at 72°C for 60 seconds. The final elongation took place at 72°C for 5 minutes. Visualization of RT-PCR results was performed by electrophoresis in 1.5% agarose gel.

3. Results and discussion
A visual examination of winter wheat fields during the tillering phase (in March in Chechnya and the Rostov region; in May in Moscow) plants with signs of mottling, chloroticity, mosaicity of leaves, as well as a general inhibition of the growth of individual plants, were found. Plant lesions were predominantly focal in nature. The visually revealed prevalence of viroses in the examined three regions did not exceed 0.5%. No insects or mites were found on the examined plants.

A reliable method for detecting and identifying viruses, incl. brome mosaic virus is a classical PCR followed by electrophoresis. Genomic BMV RNA is well studied, which made it possible to develop diagnostic primers for its various regions.

Of the 11 analyzed samples of winter wheat collected from the farms of the Rostov region, 2 samples may have been infected with the brome mosaic virus.

Figure 1 shows the results of electrophoresis. Samples 5 * and 6 * may have contained BMV RNA. For more reliable confirmation of the presence of a pathogen, the results of RT-PCR using alternative primers, as well as sequencing of amplicons, are required. In winter wheat plants collected in the Chechen Republic and in the fields of Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, the brome mosaic virus was not detected by PCR, despite clear signs of mosaic and yellowish mottling on the leaves. There are reports that in Russia the brome mosaic virus was detected in the Voronezh, Saratov and Samara regions, in the Northwest, Siberia and the Far East [14].

4. Conclusion.
The brome mosaic virus is a polyphage with high harmfulness to cereal crops. For a successful fight against the disease, it is important to know the prevalence of the virus by regions of the country. In our studies, it was detected by RT-PCR in winter wheat samples from the Rostov region. In winter wheat plants from the Chechen Republic and the Moscow region (Russian State Agrarian University - Moscow Timiryazev Agricultural Academy), we did not detect the brome mosaic virus, although there were
virus-like symptoms on the leaves. To protect against viral diseases of cereals, along with chemical means of protecting plants from insect vectors, it is necessary to pay attention to biological, as well as selection and genetic methods, as an important element of integrated protection against pathogenic complexes. Accurate identification of pathogens is required to select and develop virus-resistant varieties. In addition, the development of reliable methods for the diagnosis of viruses in cereal crops is necessary to meet the phytosanitary requirements set by importing countries, given that the Russian Federation ranks first in the export of grain in the world.

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