DIAGNOSTICS OF SEED-BORNE CEREAL VIRUSES IN AGROECOSYSTEMS OF UKRAINE

Using various complementing diagnostics techniques, we have analyzed spread of cereal viruses capable of seed transmission for Ukraine. Testing different cultivars and lines of cereal plants massively cultivated in Ukraine showed that seed(s) of 10 cultivars (11.8% of their total quantity) contains Barley stripe mosaic virus (BSMV).

Key words: cereal viruses, seed transmission, barley stripe mosaic virus.

Introduction. Conservation of a virus in a seed for subsequent virus transmission is an ingenious strategy for virus survival because the seed virtually links sowing seasons. This approach is of special importance for viruses having narrow host range and for viruses which are not readily transmitted by vectors. For cereals, barley stripe mosaic virus (BSMV) is a showcase of probably most specialized virus as the seed transmission is vital for its survival [1; 2; 3]. In addition to BSMV it is known that wheat streak mosaic virus (WSMV) may also be transmitted with small seed transmission rate of the virus as 1% (i.e., when concurrent bacterial and fungi infections. WSMV and BSMV were detected using DAS-ELISA with commercial polyclonal test systems (Loewe Biochemica, Germany) induced losses are normally due to flower sterility (BSMV is transmitted by vectors. For cereals, barley stripe mosaic virus (BSMV) is a showcase of probably most specialized virus as the seed transmission is vital for its survival [1; 2; 3]. In addition to BSMV it is known that wheat streak mosaic virus (WSMV) may also be transmitted with small seed transmission rate of the virus as 1% (i.e., when concurrent bacterial and fungi infections. WSMV and BSMV were detected using DAS-ELISA with commercial polyclonal test systems (Loewe Biochemica, Germany)

BSMV-infected plants produce 20-50% less seed, mainly due to the decrease of the number productive stems and number of seeds in a spike. BSMV is spread worldwide where cereals are grown. The virus cannot be inactivated by chemical or temperature seed treatment (in spite of the fact that temperature point for virus inactivation is 70°C [2; 3].

Starting from the 1960ies, many authors described diseases of cereal crops induced by WSMV and BSMV in Ukraine. Main foci of research were biological properties of these viruses, their spread, visual appearance of the diseases on various cultivars, harmfulness, etc. [7; 8; 9]. Today, however, these pathogens (and especially seed transmission) is totally neglected. BSMV is a good example of the virus which spread remains unknown.

This work was aimed at analyzing spread of WSMV and BSMV in Ukrainian agroecosystems using different diagnostic techniques, and also at testing plant selection material of major cereal crops (available at the Bank of plant genetic resources of Ukraine) for BSMV infection.

Materials and methods. For obtaining reliable data on detection and spread of seed-borne cereal viruses we have conducted 10-year monitoring of wheat and barley commercial sowings showing symptoms typical for these pathogens. The monitored areas were Vinnytsya, Dnipropetrovsk, Kyiv, Lviv, Mykolayiv, Odessa, Poltava, Kher son, Kmelnytskyi and Cherkassy regions. During the visual assessment of the fields attention was paid to the percentage and relative spread of diseased plants, to occurrence of insect vectors, and to the abundance of concurrent bacterial and fungi infections. WSMV and BSMV were detected using DAS-ELISA with commercial polyclonal test systems (Loewe Biochemica, Germany) following the manufacturer’s recommendations. Samples with optical density of 0.2 and higher were considered positive in ELISA [10].
For direct virus detection, study of their morphological properties and dimensions transmission electron microscopy (TEM) was used. For microscopy, plant samples were homogenated in 0.1 M PBS, pH 7.4, and centrifuged at 4,000 rpm for 15 min. The supernatant was deposited on Formvar-coated copper grids further contrasted with 2% uranil acetate for 10 min.

For detection contamination of cereal seed bank selection material with viruses, seedlings and young plants (stage of 4 leaves) of 85 cultivars and lines were used. 10 plants of each genotype were tested for viruses using ELISA as described above.

**Results and discussion.** WSMV is demonstrated to be one of the most spread plant viruses in agroecosystems of Ukraine. By means of visual diagnostics and ELISA, we have detected WSMV in winter and spring wheat, winter and spring barley in sowings from Vinnytsya, Dnipropetrsivsk, Kyiv, Odessa, Poltava, Kharkiv and Cherkassy regions. WSMV has been most widely spread in central, northern and eastern parts of Ukraine. Season of 2007/2008 yy has shown a peak in virus spread, as well as warm and humid autumn of 2012 (especially in northern and eastern regions). Different cultivars developed varying symptoms of WSMV infection ranging from small streak mosaics to light green stripe mosaics, even when grown on the same field. ELISA confirmed that plants with differing symptoms have been infected with the same virus, WSMV, in the form of monoinfection. Importantly, plants with stripe mosaic symptoms were grouped together forming a focus of infection in the field (Fig.1a), when separate plants with streak mosaic symptoms were more or less evenly distributed (Fig.1b).

Fig.1. WSMV symptoms of winter wheat plants: (a) late autumn, Kharkiv region; (b) late spring, Kyiv region

In our opinion, these results may indicate different means of virus transmission in the field. Separate diseased plants were probably germinated from WSMV-contaminated seed, when in case of foci of infection the virus was rather vector-transmitted as *Aceria tritici* mites were found abundant in the leaves' sulci.

BSMV has been detected only sporadically on winter wheat in 2003 (Kyiv and Poltava regions), in 2006 (Vinnytsya region), and in 2008 (Kyiv region). This probably is connected to the unique mean of virus transmission by seed. In addition, BSMV-contaminated seed has low mass and normally is not used for sowing.

Microscopy analysis confirmed plants' infection with either WSMV or BSMV. We have detected typical particles of *Tritimovirus* genus, *Potyviridae*, 700x13-14 nm (Fig.2a), and rod-shaped particles of 120-150x20 nm typical for *Hordeivirus* genus (Fig.2b). ELISA confirmed the TEM results.

We have also tested cereal seed bank selection material for BSMV and demonstrated that 10 cultivars (11.8% of their total quantity) contained BSMV. These were spring wheat cultivars "Kharkivska 30", "Rannya 93", "Prohorovka", "Voronezhska 6", "Saratovska 60", and "Saratovska 68"; winter wheat cultivar "Skala"; winter barley cultivar "Avangard"; selection lines "D-253"and "D-257".

This information must be taken into account when breeding new varieties.

Fig.2. Electron micrography of WSMV (a) and BSMV (b) (x25000)

We need to say that various cultivars and lines of cereals demonstrated differing rate of BSMV infection. "Rannya 93", "Prohorovka", and "Saratovska 68" cultivars were most contaminated (45-65 %), when "Voronezhska 6" cultivar and lines "D-253"and "D-257" were characterized with only 10% of infection (Fig.3).
The absolute number of initially infected seeds is vitally important for disease progression, especially in case of selection/breeding material. These results underline the need for careful testing of source genetic material.

**Conclusions.** We have analyzed agriecosystems in 11 cereal-growing regions of Ukraine for occurrence and spread of seed-borne viruses. Using different diagnostic approaches we have demonstrated significant spread of BSMV. Wheat streak mosaic virus – the pathogen gathering its epidemic potential in many regions of the country. Moreover, we have also detected barley stripe mosaic virus in several agriecosystems and breeding material of major cereal cultures provided by the Bank of plant genetic resources of Ukraine. BSMV is highly specialized for seed transmission with unknown vectors. It’s also spread mechanically by contact. BSMV is transmitted by seed of only susceptible/tolerant barley cultivars. The efficiency of its seed transmission by resistant cultivars is negligible [12, 13].

Hence, co-adaptation of the virus and the host favors seed transmission of BSMV. Mild strains of this virus are more readily seed-transmitted and do not induce severe visual symptoms. The symptoms depend on the growing conditions, underlying the need for careful monitoring of virus spread.

**Fig.3. Percentage of seed contamination with BSMV for different cultivars and lines of cereals**

**References**


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**ДІАГНОСТИКА ВІРУСІВ ЗЕРНОВИХ КУЛЬТУР, ЩО ЗДАТНІ ДО НАСІННЄВОЇ ПЕРЕДАЧІ, В АГРОЕКОСИСТЕМАХ УКРАЇНИ**

Використовуючи різні методи діагностики визначено поширення вірусів зернових культур, що здатні до насінневої передачі в агроекосистемах України. Тестування сортів та ліній зернових, які вирощуються в Україні, показало, що насіння лише 10 (11,8 %) сортів контаміноване вірусом штрихуватої мозаїки ячменю (ВШМЯ).

Ключові слова: вірус зернових культур, насіннева передача, вірус штрихуватої мозаїки ячменю.

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Іспользуя различные методы диагностики определено распространение вирусов зерновых культур, способных к семенной передаче в агрокосистемах Украины. Тестирование сортов и линий зерновых, которые произрастают в Украине, показало, что семена только 10 (11,8 %) сортов контаминаированы вирусом штрихуватой мозаики ячменя (ВШМЯ).

Ключевые слова: вирусы зерновых культур, передача семенами, вирус штрихуватой мозаики ячменя.
OCCURRENCE OF CUCUMBER MOSAIC VIRUS IN VEGETABLE CROPS IN UKRAINE

Symptomatic plants of Cucurbitaceae and Solanaceae families collected in Ukrainian agriecosystems used for commercial cultivation of vegetables have been analyzed. According to the ELISA results, 38 samples (of 126 samples in total, i.e., 30%) have been infected with Cucumber mosaic virus. CMV is widespread in Vinnytsia, Zaporizhzhia, Kyiv, Odessa, Poltava and Cherkasy regions. We have obtained a cDNA of 500 bp corresponding to the coat protein gene of Ukrainian CMV isolate.

Key words: Cucurbitaceae and Solanaceae families collected, ELISA.

Introduction. Viral infections cause considerable economic losses to farms engaged in vegetable growing. Usually large-scale assessment of such damages is conducted visually. However, the similarity of symptoms on plants caused by pathogenic agents of different nature, such as viruses, bacteria, fungi, in practice makes impossible to use effective means of treatment and prevention without well-defined identification of pathogens [1].

The only efficient way of controlling viral diseases is their timely diagnostics and putting in place respective preventive measures with regard to eliminate vectors and reservoirs of viral antigens, introducing resistant cultivars of plants and obtaining virus-free material [2].

Cucumber mosaic virus (CMV) belongs to genus Cucumovirus of family Bromoviridae. CMV infects 200 species of plants belonging to 60 families, but most known for damaging cucumbers cultivated in the open field conditions [3]. CMV cause severe symptoms on plants and fruits, and greatly reduces the yield of the pumpkin crops. In recent years new data confirmed the expansion of this viral disease onto new territories together with spread of fruits, and greatly reduces the yield of the pumpkin crops.

Visual observation of external symptoms is an unreliable method for detection and identification of viral infection, because the appearance of viral infection mainly depends on interaction between a virus and a host. Besides, the strains of the same virus can often cause a variety of symptoms changing from hypersensitive to asymptomatic reaction on plants of the same species. The growing conditions and the presence of mixed infection can also effect the development of symptoms. For example, sometimes only mixed infection of pepper may lead to the appearance of mosaics and motting. That's why the diagnosis of viral infection should be confirmed by specific methods of examination and identification of viruses, particularly by serological tests.

Results and discussion. Approximately 126 plant samples belonging to the Cucurbitaceae and Solanaceae families were selected and tested for CMV. Plant samples were collected from following regions of Ukraine: Autonomous Republic of Crimea, Vinnytsia, Zaporizhzhia, Kyiv, Kirovohrad, Odessa, Poltava, Cherkasy and Chernihiv regions. Plants of Cucurbitaceae family (cucumber, squash, pumpkin, and zucchini) showed puckering, distortion, vein banding, yellowing, filamentary, yellow mosaic on leaf blade; dark green spots of different size, knobs and malformations on fruits (Fig. 1). Plants of Solanaceae family (tomato, pepper, eggplant) showed disease symptoms in month after seedtime during flowering. The first symptoms were yellow spots and vein clearing on young leaves followed by systemic yellow and green mosaics, chloroses and local necroses (Fig. 1).

The purpose of the work was to establish the distribution of Cucumber mosaic virus in vegetable crops in Ukraine.

Materials and methods. The samples were selected following the visual examination of virus symptoms. For virus detection, plant material was homogenized in 0.1 M phosphate buffered saline (PBS), pH 7.4, 1:2 (m/v). Plant components were removed by centrifugation at 5,000 g for 20 minutes.

DNA extraction was carried out using RNeasy Plant Mini kit (Qiagen, UK). The results were checked by electrophoresis of nucleic acids in 1.5% agarose gel. Reverse transcription reaction (RT-PCR) was performed using primers specific to the coat protein gene of CMV:

- forward primer – 5’TATGATAAGAAGCTTGGTTTCCCGCA-3’
- reverse primer – 5’TTTTAGCCGTAAGCTGGATGGACAACC-3’

PCR products were analyzed by electrophoresis in 1.5% agarose gel using markers Gene Ruller 100 bp DNA Ladder plus (Fermentas, USA) [7].