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БАКТЕРИОФАГИ ЗІ ЗРАЗКІВ РОСЛИН *PULSATILLA PRATENSIS* ПРИРОДНОЇ ФЛОРИ УКРАЇНИ

При дослідженні зразків з рослин Pulsatilla pratensis, відібраних на території Канівського природного заповідника, було виділено 6 бактеріофагів з довгими хвостовими відростками, що відрізнялися за розмірами, біологічною (літичною) активністю та білковим складом.
Ключові слова:бактеріофаги, флора України, Pulsatilla pratensis.

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БАКТЕРИОФАГИ ИЗ ОБРАЗЦОВ РАСТЕНИЙ *PULSATILLA PRATENSIS* ПРИРОДНОЙ ФЛОРЫ УКРАИНЫ

При исследовании образцов из растений Pulsatilla pratensis, отобранных на территории Каневского природного заповедника, было выделено 6 бактериофагов с длинными хвостовыми отростками, которые отличались по размерам, биологической (литической) активностью и белковому составу.
Ключевые слова: бактериофаги, флора Украины, Pulsatilla pratensis.

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DIAGNOSTICS OF SEED-BORNE CEREAL VIRUSES IN AGRIECOSYSTEMS 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 seeds with the rate of 0,1-0,2% for maize [4] and 0,5-1,5 % for different wheat genotypes [5]. In the early 2000ies, seed transmission has brought WSMV from the USA to Australia where this virus (having probably adopted to a new vector species, *Aceria tosichella*) induced heavy epidemics [6]. Different authors pointed that even such small seed transmission rate of the virus as 1% (i.e., when 1% of seeds contain virus) may lead to multiple virus infection of plant generation (105-107 infected plants per hectare). This is why annual yield losses of cereals attributed to WSMV in the North America make approximately 5%. However, local outbreaks favored by intensive virus spread by *Aceria tritici* at the early stage of plant growth may lead to total loss of the crop yield [1].

As regarding BSMV infection in the USA, natural virus infection typically leads to barley yield losses of 30-31%. Virus induced losses are normally due to flower sterility (BSMV is transmitted by both seeds and pollen) [2]. Efficiency of seed transmission of this virus depends on virus strain, stage of plant growth at which it became infected, and also on species and cultivar of the crop. Available data suggest that BSMV retains its infectivity even in the seeds stored for more than 19 years [2]. This fact is of importance when choosing material for plant selection aiming at breeding new virus-resistant cereal crops. In addition it's worth to say that 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 agriecosystems 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, Dnipropetrivsk, Kyiv, Lviv, Mykolayiv, Odessa, Poltava, Kherson, Kmelnytskiy 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 Formvare-coated copper grids further contrasted with 2% uranil acetate for 10 min [11].

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 agriecosystems 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, Dnipropetrivsk, 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).

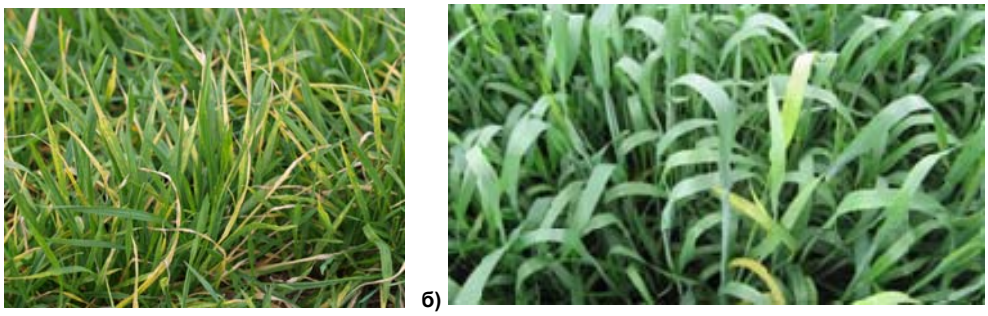

Fig.1. WSMV symptoms of winter wheat plants: (a) late autumn, Kharkiv region; (b) late spring, Kyiv region

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.

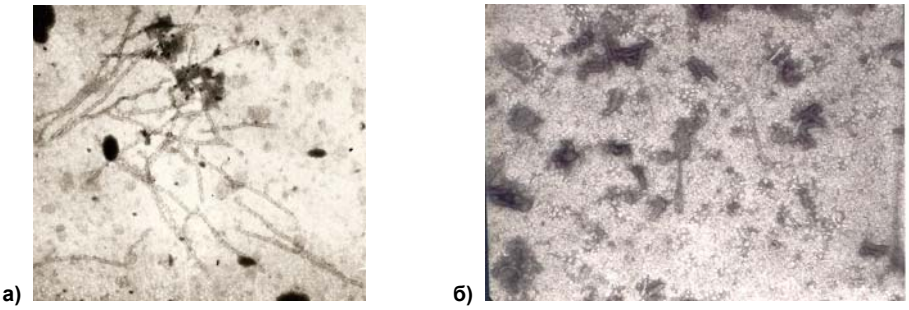

Fig.2. Electron micrography of WSMV (a) and BSMV (b) (x25000)

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).

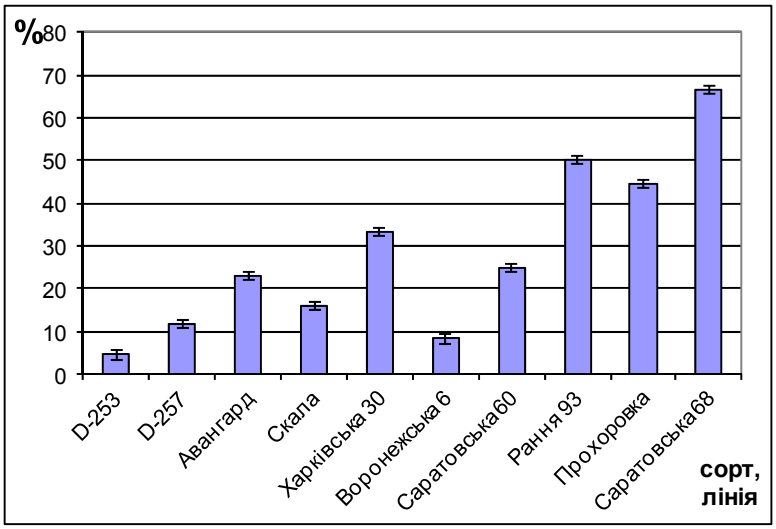

Fig.3. Percentage of seed contamination with BSMV for different cultivars and lines of cereals

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 *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 i*n 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.

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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 насіннєвої 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:віруси зернових культур, насіннєва передача, вірус штрихуватої мозаїки ячменю.

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ДИАГНОСТИКА ВИРУСОВ ЗЕРНОВЫХ КУЛЬТУР, КОТОРЫЕ ПЕРЕДАЮТСЯ СЕМЕНАМИ, В АГРОЭКОСИСТЕМАХ УКРАИНЫ

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

Ключевые слова: вирусы зерновых культур, передача семенами, вирус штриховой мозаики ячменя.