sample of *Chlorococcum sp*. culture we have identified spherical virus (or virus-like) particles of 50±2nm in diameter (Fig.2). However we have been able to demonstrate presence of virus particles in the lysates of other algae cultures. We deem that this is connected with low concentration of the particles in the sample not allowing their easy isolation and concentration.

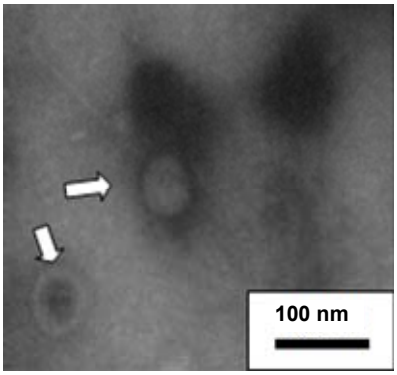

Fig.2. TEM image of virus-like particles in the autolysate sample of *Chlorococcum sp.* algae culture

Further studies will involve analysis of new water samples, as well as isolation and concentration of identified virus isolates.

In conclusion, biotesting assay of water samples collected in Verbne and Velykyi Opechen lakes, and from Rylskyi Park' lakes (Kyiv) were able to lyse *Planophila sp*. and *Chlamydomonos heterogama* algae test cultures. Respectively, water samples collected from the reservoir near the city of Slavutych and from Siverka River near Vita-Pochtova village lysed algae test cultures of *Chlorella sp*. and *Radiosphaera sp*. Using differential centrifugation of the samples, we purified and concentrated virus isolate(s) from the samples of autolysis of *Carteria crucifera* and *Chlorococcum sp*. algae cultures. TEM studies of such virus isolate(s) confirmed presence of spherical virus (or virus-like) particles of 50±2nm in diameter. Further work will be focused on studying molecular properties of the virus(es).

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Надійшла до редколегії 30.11.10

UDC 632.38

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SOIL-BORNE VIRUSES DETECTED IN SUGAR BEET IN LITHUANIA AND UKRAINE

Проведено обстеження зразків цукрового буряку отриманих на території Литви та України на наявність вірусу некротичного пожовтіння жилок буряку (ВНПЖБ), ґрунтового вірусу буряку (ҐВБ) та Q вірусу буряку (ВБQ). Тестування проводили методами ІФА, в модифікації DAS і TAS, та мультиплексною ЗТ-ПЛР (мЗТ-ПЛР), що дозволяє одночасно ідентифікувати ВНПЖП, ҐВБ та ВБQ. Зразки коренеплодів цукрового буряку, з симптомами ризоманії, відбирали на плантаціях Литви та України та тестували на наявність ВНПЖБ за допомогою DAS-ELISA. Для детекції ҐВБ, методом TAS-ELISA, використовували лише ті зразки, що позитивно прореагували з антисироваткою до ВНПЖБ. У литовських зразках цукрового буряку не було відмічено випадків змішаної інфекції ВНПЖБ і ҐВБ або ВПQ. На противагу в українських зразках (відібраних в Рівненській, Львівській, Івано-Франківській та Хмельницькій областях) крім ВНПЖБ була доведена наявність ҐВБ. Ідентифікацію ҐВБ підтверджували за допомогою мЗТ-ПЛР, в усіх випадках були отримані специфічні продукти для ВНПЖБ (545 п.о.) та ҐВБ (399 п.о.). ВПQ не було виявлено в жодному з досліджених зразків.

Tests for detection Beet necrotic yellow vein virus (BNYVV), Beet soil-borne virus (BSBV) and Beet virus Q (BVQ) in sugar beet samples from Lithuania and Ukraine were conducted using direct DAS-ELISA, direct TAS-ELISA and multiple RT-PCR (mRT-PCR), allowing simultaneous identification BNYVV, BSBV and BVQ. Sugar beet root samples with suspicious symptoms of rhizomania were collected in Lithuanian and Ukrainian sugar beet growing areas and analyzed by DAS-ELISA for the presence of BNYVV. For BSBV detection by TAS-ELISA only samples with positive reaction to BNYVV were used. Mixed infections of BNYVV and BSBV or BVQ in sugar beet samples in Lithuania were not detected. Meanwhile, in some Ukrainian sugar beet samples (from Rivno, Lviv, Ivano-Frankivsk and Khmelnytskyi regions) along with BNYVV, presence of BSBV was detected. Identification of BSBV was confirmed by mRT-PCR. In all cases specific BNYVV (545 bp) and BSBV (399 bp) products were obtained. BVQ was not found in any of investigated samples.

Introduction. Several soil-borne viruses are known to infect sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) worldwide. Rhizomania, caused by *Beet necrotic yellow vein virus* (BNYVV; genus *Benyvirus*) (Tamada and Baba, 1973), is one of the most destructive diseases of sugar beet. Losses of sugar beet root yield can reach 50-60 % (Henry, 1996). Rhizomania was originally described in Italy (Canova, 1959) but now it is present in sugar beet areas all over the world. BNYVV has a multipartite single-stranded RNA genome with all natural isolates containing four RNA species, although some isolates have a fifth RNA (Tamada et al., 1989). The larger RNA1 and RNA2 contain the housekeeping genes of the virus and are always required for infection, whereas the smaller RNAs are involved in pathogenicity and vector transmission. RNA5-containing isolates are restricted to Asia and some parts of Europe and these isolates tend to be more aggressive (Richards and Tamada, 1992; McGrann et al., 2009). BNYVV is transmitted and preserved in the soil by *Polymyxa betae* Keskin. Like BNYVV, the plasmodiophorid has a host range mainly restricted to the roots of *Chenopodiaceae* spp. (Barr and Asher, 1992) and is present in most sugar beet growing countries (Payne and Asher, 1990). The virus can survive in *P. betae* cystosori for more than 15 years (Abe and Tamada, 1986; McGrann et al., 2009). According to the alert list of European and Mediterranean Plant Protection Organization (EPPO), BNYVV has been detected in many European, Asian countries (Austria, Belgium, Bulgaria, China, Croatia, Czech Republic, Denmark, Federal Republic of Yugoslavia, France, Germany, Greece, Hungary, Iran,

Japan, Kazakhstan, Kyrgyzstan, Mongolia, Poland, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, The Netherlands, Turkey and the United Kingdom) (Kanzawa and Ui, 1972; Gao et al., 1983; Asher, 1993; Miyanishi et al., 1999; Lennefors et al., 2000; Nielsen et al., 2001; Mouhanna et al., 2002; Sohi and Maleki, 2004; OEPP/EPPO, 2005; Yilmaz et al., 2007), also in Morocco and North America (USA) (Al Musa and Mink, 1981; OEPP/EPPO, 2005), and is likely to continue its spread across the world (Rush et al., 2006; McGrann et al., 2009).

Other soil-borne viruses detected in sugar beet are *Beet soil-borne virus* (BSBV) and *Beet virus Q* (BVQ) that belong to the genus *Pomovirus* and have multipartite genomes consisting of three RNA species (Hutchinson et al., 1992; Kaufmann et al., 1992; Koenig et al., 1998). BVQ was originally considered to be a serologically distinct strain from BSBV, but finally Koenig (1998) proved its different RNA composition substantiating its separation from BSBV. BSBV was first found by Ivanovič and Macfarlane (1982) in England and further described by Henry et al. (1986). It is widespread in sugar beet growing areas all over the world. BSBV has been detected in Europe (United Kingdom, Germany, Sweden, The Netherlands, Spain, France, Italy, Finland, Belgium, Bulgaria, Hungary, Turkey, Slovakia, Denmark, Poland and China) (Henry and Jones, 1986; Lesemann et al., 1989; Lindsten, 1993; Bremer et al., 1990; Meunier et al., 2003; Šubíková, 1995; Danielsen et al., 1992; Borodynko et al., 2006; Wang et al., 2007), in the Middle East (Iran and Syria) (Farzadfar et al., 2002; Mouhanna et al., 2002) and also in the USA (Lindsten and Rush, 1994). BVQ has been detected in Belgium, Bulgaria, Czech Republic, France, Germany, Hungary, Italy, Poland, Sweden and The Netherlands (Meunier et al., 2003; Rysanek et al., 2006; Borodynko et al., 2009).

Harmfulness of these two pomoviruses is questionable, because they also occur in fields with no problems concerning sugar beet growing. In common with BNYVV, the two pomoviruses have a common vector (the soil-borne protist *P. betae*), similar host ranges and particle morphologies but differ in serological properties, genome structure and sequence (Koenig et al., 1998; Koenig and Lesemann, 2005). BSBV and BVQ often occur together in the same field and, not rarely, in rhizomania-affected sugar beet plants (Meunier et al., 2003), although their etiological role in the disease remains a matter of debate (Prillwitz and Schlosser, 1992).

Surveys to detect BNYVV have been regularly carried out since 1998 in Lithuania (Lithuanian State Plant Protection Service) and since 1997 in Ukraine (Institute for Sugar Beet Research of NAAS, Ukraine). In recent years rhizomania was identified in three areas of Lithuania (Jackeviciene et al., 2005; Žižytė, et al. 2006; Zizyte and Staniulis, 2007). From 1997 till 2003 rhizomania was also detected in 4 areas of Crimea and 17 regions of Ukraine (Nurmukhammedov, 2005).

The aim of this work was to detect mix infection of beet soil-borne viruses in Lithuanian and Ukrainian sugar beet growing areas.

Materials and methods. Sugar beets with suspicious symptoms of rhizomania were collected in different regions of Lithuania and Ukraine. Samples of rootlet tissue were used for ELISA analysis and total RNA extraction. BNYVV was tested by double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) and BSBV was tested by triple antibody sandwich-ELISA (TAS-ELISA) using Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) immunological kits as described by Clark and Adams (1977). Absorbance (A_{405}) was recorded using a Multiskan RC microplate reader 1-2 h after adding the substrate (*p*-nitrophenylphosphate). The ELISA test was considered positive if absorbance of the investigated sample was greater than 3 times the absorbance of the negative (healthy plant) control. Total RNA extraction was performed using QuickPrep total RNA extraction kit for the direct isolation of total RNA from most eukaryotic tissues or cells (Amersham Bioscience) or TRIzol Reagent (Invitrogen) according to the kit protocol. Three pairs of primers (Meunier et al., 2003) were combined in the mRT-PCR for specific BNYVV, BSBV and BVQ products (545 bp, 399 bp and 291 bp) amplification. The primers used in mRT-PCR are listed as follows (5'-3'): BNYVV/for, ACATTTCTATCCTCCTCCAC and BNYVV/rev, ACCCCAACAAACTCTCTAAC for RNR2; BSBV/for, CTTACGCTGTTCACTTTTATGCC and BSBV/rev, GTCCGCACTCTTTTCAACTGTTC for RNR1; BVQ/for, GCTGGAGTATATCACCGATGAC and BVQ/rev, AAAATCTCGGATAGCATCCAAC for RNR2. For each RT reaction, 20 Mμl of each reverse primers were mixed with 3 μl of total RNA and 3 μl of diethyl pyrocarbonate (DEPC)-treated water. The mixture was incubated at 70° C for 5 min and chilled on ice prior to the addition of 1.5 μl of DEPC-treated water, 2 μl of dNTPs (10 mM), 4 μl of MMLV RT 5× buffer (MBI Fermentas), 0.5 μl Ribonuclease Inhibitor (40 U/ μl) (MBI Fermentas) and 1 μl of MMLV reverse transcriptase (200 U/ μl) (MBI Fermentas). For the PCR, 20 Mμl of each of the forward and reverse primers was added to 12 μl of DEPC-treated water, 4 μl of $MgCl_2$ (25 mM) (MBI Fermentas), 6 μl of *Taq* polymerase 10× buffer (MBI Fermentas), 1.5 μl of dNTPs (10 mM), 0.5 μl of *Taq* polymerase (5 U/ μl) (MBI Fermentas), and 4 μl of cDNA. Amplification cycles were as follows: a first denaturation for 3 min at 94° C and then 35 cycles of denaturation for 30 s at 94° C, annealing for 30 s at 63° C, and elongation for 2 min at 72° C. A final elongation for 7 min at 72° C was added. In PCR reactions healthy plant material and water controls were used. All PCR products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide and visualized under UV light.

Results and discussion. In 2009 sugar beet root samples with suspicious symptoms of rhizomania were collected in 14 Lithuanian (Kaunas, Kėdainiai, Marijampolė, Šakiai, Vilkaviškis regions) and 17 Ukrainian sugar beet growing fields (Rivno, Lviv, Ternopil, Ivano-Frankivsk, Chernivtsi, Khmelnytskyi, Vinnytsya and Zhytomyr regions) and analyzed by DAS-ELISA for the presence of BNYVV. For BSBV detection by TAS-ELISA only samples with positive reaction to BNYVV were used. BVQ detection was relied on mRT-PCR, allowing simultaneously identify BNYVV, BSBV and BVQ viral particles in the same sample (Meunier et al., 2003). According to the positive DAS-ELISA results BNYVV was proved not to be very widespread in Lithuanian sugar beet growing areas because it was present just in 1 region (central part of Lithuania, Kaunas region) (*data not shown*). In Ukraine rhizomania was confirmed in 10 sugar beet growing areas. Mixed infections of BNYVV and BSBV or BVQ were not detected in Lithuanian sugar beet samples (*data not shown*). Meanwhile, in some Ukrainian sugar beet samples mix infection of BNYVV and BSBV was found (Table 1). BSBV detection was carried out by both TAS-ELISA and mRT-PCR. Obtained ELISA results of BNYVV and BSBV mix infection are shown in Table 1. According to TAS-ELISA results BSBV was detected in ukr2, ukr4, ukr9, ukr10 and ukr11 (Rivno, Lviv, Ivano-Frankivsk and Khmelnytskyi regions) sugar beet samples (Table 1.). Identification of BSBV was also confirmed by mRT-PCR. Three specific PCR primer pairs (Meunier et al., 2003), amplificating 291 bp (BVQ), 399 bp (BSBV) and 545 bp (BNYVV) products were used for

mRT-PCR. In all cases specific BNYVV (545 bp) and BSBV (399 bp) products were obtained (Fig. 1.). mRT-PCR confirmed the ELISA results. BVQ was not found in any of investigated samples although it is known, that BVQ infection was previously reported in Ukrainian agrocenosis (Senchugova et al., 2007).

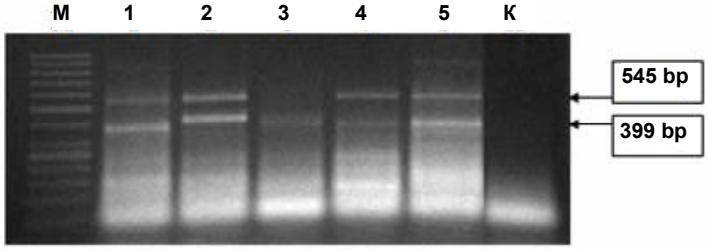

Fig. 1. mRT-PCR products of investigated mix infection of BNYVV and BSBV in 2% agarose gel: M – GeneRuler 50 bp DNA Ladder; 1 – ukr2 (Rivno); 2 – ukr4 (Lviv); 3 – ukr9 (Ivano-Frankivsk); 4 – ukr10 (Khmelnytskyi); 5 – ukr11 (Khmelnytskyi); K – water control

Table 1. **ELISA results of investigated mix infection of BNYVV and BSBV in Ukrainian sugar beet samples**

Location	Isolate	Virus infection	
		BNYVV	BSBV
		DAS-ELISA, absorbance, 405 nm	TAS-ELISA, absorbance, 405 nm
Rivno region, Radyvyliv area, Krupets village	Ukr2	0.586 +	0.458 +
Lviv region, Brody area, Brody	Ukr3	0.561 +	0.27 –
Lviv region, Radechiv area, Chmilno village	Ukr4	0.848 +	0.342 +
Lviv region, Radechiv area, Babychi village	Ukr5	1.011 +	0.249 –
Lviv region, Busk area, Ozhydiv village	Ukr6	0.452 +	0.253 –
Ternopil region, Chortkiv area, Oryshkivtsi village	Ukr7	0.451 +	0.278 –
Ternopil region, Zalischyky area, Torske village	Ukr8	0.466 +	0.288 –
Ivano-Frankivsk region, Gorodenka, Yaseniv-Pilnyi village	Ukr9	0.674 +	0.423 +
Khmelnytskyi region, Starokostyantyniv area, Starokostyantyniv	Ukr10	0.705 +	0.345 +
Khmelnytskyi region, Starokostyantyniv area, Ladygy village	Ukr11	0.843 +	0.512 +
K –		0.151	0.176
K +		1.65	2.408

"K–" – negative control; "K+" – positive control; "–" – negative result; "+" – positive result. The ELISA test was considered positive if absorbance of the investigated sample was greater than 3 times the absorbance of the negative (healthy plant) control (Clark and Adams, 1977).

Acknowledgments. This work was supported by the grant from Research Council of Lithuania No TAP-48/2010 and Lithuanian Ministry of Education and Science No TAP-34/2010.

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Надійшла до редколегії 29.11.10

UDC 578.85/.86

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ELECTRON MICROSCOPY DETECTION OF VIRUSES IN THE CACTUS COLLECTION OF UKRAINE

Проведено скринінг колекцій кактусових у ботанічних садах України. У всіх обстежених колекціях кактуси були інфіковані вірусами. Виявлено вірус, який за морфологічними характеристиками подібний до вірусу кактусів 2.

Screening of Cactaceae plants on virus diseases in the collections of Ukrainian Botanical gardens have been conducted. Cactus plants in all collections are virus infected. Basing on morphological properties detected virus is related to Cactus virus 2.

Introduction. Cactus collections in Ukrainian botanical gardens are represented by hundreds various types of plants, located in the limited space greenhouses. This situation leads to rapid mechanical transmission of pathogens. Exchange collection material without testing on virus infection, can increases chances of uncontrolled distribution of viral infections. In case of asymptomatic virus infection in cactus uncontrolled import to Ukraine succulents makes a danger because these plants can become the source of distribution pathogens in collections. In addition, cactuses could support reproduction of viruses other types of plants and, thus, be the reservoirs of plant virus infections. A timely exposure and permanent control of the state of population of these cultural plants is the obligatory link of the system of their protecting from this group of pathogens.

Materials and methods. As biological material were used plants with the signs of affection: *Opuntia* sp., *Opuntia microdaysys* v. rufida, *Consolea rubenscens*, *Pereskia aculeata* v. godseffiana, *Echinocereus* sp., *Caralluma* sp., *Consolea rubescens, Pseudolobivia ancistrophora, Lophophora williamsii, Echinocereus penthalophus, Ferocactus echidne, Lophophora echinata, Rhipsalis* sp., *Rhipsalis pachyptera, Pseudolobivia cojasii* and crested graft plants *Chamaereus silvestrii*, *Echinopsis* sp., with virus-like symptoms from greenhouse collections of Fomin' Botanic Garden of Taras Shevchenko' Kyiv national university (Kyiv), *Mamillaria centricirrha, Trichocereus bridgesii, Ritterocereus prinosus* – from the collection of Karazin' Botanic Garden of Kharkiv national university (Kharkiv)), *Ferocactus echidne, Gymnocalycium sp. Opuntia sp*. – from the collection of Botanical garden of Ivan Franko' Lviv national university (Lviv*)*), *Opuntia* sp., *Opuntia arechavelerae* – from the collection of the Donetsk botanical garden of the National academy of sciences of Ukraine (Donetsk) and *Opuntia* sp., *Opuntia microdaysys* v. *alba* – from the collection of Botanical garden of I.I. Mechnikov Odesa national university (Odesa).

Morphology of found viruses was determined by the method of transmission electron microscopy. Plants homogenized with addition of 0,1M phosphate buffered saline (pH 7,4). For besieging of cellular admixtures conducted low-speed centrifugation 7000 rpm during 20 minutes.

Preparations inflicted on copper string-bags from 0,2% formvar lining. The negative contrasting was conducted by 2% uranilacetat during 1,5 min. The revision of standards was conducted in an electron microscope EM–125 (Sumi, Ukraine), with an instrumental increase 30 000.

Results and discussion. Virus diseases of *Cactaceae* plants aren't enough studied. The most widespread viruses affecting plants of this family are *Cactus virus X* (genus *Potexvirus,* family *Alphaflexiviridae*): virions filamentous, not enveloped; usually flexuous with clear modal length 520 nm and 13 nm wide; *Zygocactus montana X virus* (genus *Potexvirus,* family *Alphaflexiviridae*): virions are flexuous with clear modal length 519 nm and 10 nm wide; *Cactus mild mottle virus* (genus *Tobamovirus*): virions long 320 nm and 18 nm in diameter, *Sammons' Opuntia virus* (genus *Tobamovirus*) virions rod-shaped, not enveloped, long 317 nm and 18 nm wide; *Saguaro cactus virus* (genus *Carmovirus,* family *Tombusviridae*): virions not enveloped, isometric nucleocapsid, 32 nm in diameter; *Virus cactus 2* (genus *Carlavirus,* family *Betaflexiviridae*): filamentous virions with normal length 650-655 nm and 11-13 nm wide [2, 3].

At the visual inspection of cactus collections we pay attention on plants with the changes of colouring, in particular by the inlaid and chlorotic spots. In cactus collection of Fomin' Botanic Garden of Taras Shevchenko' Kyiv national university we found symptoms of necrosis, mosaic and chlorotic spots, wrinkling and atrophy of stems. The chlorotic colouring and atrophy of stems (symptoms of witches'-broom) were observed only on the *Opuntia* plants. These symptoms on *Opuntia* could be associated with phytoplasma [1]. Although we selected these plants to detect viruses as we proposed that affection of phytoplasma could reduced plant resistance to viruses. Furthermore in this situation activation of latent virus infection and following development of symptoms could took place. It should be noted that different cactus species had diverse symptoms. In collection of Karazin' Botanic Garden of Kharkiv national university we