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PHYLOGENETIC ANALYSIS OF SEED-TRANSMITTED ISOLATE OF ZUCCHINI YELLOW MOSAIC VIRUS

Zucchini yellow mosaic virus (ZYMV) remains one of the most widespread and destructive viruses affecting plants from Cucurbitaceae family in Ukraine as well as in other countries. ZYMV during the early stages of plant development can cause significant losses in yield. In current project the possibility of seed transmission of Ukrainian ZYMV isolates was tested on Cucurbita pepo plants in insect-free greenhouse. The rate was assessed by ELISA and RT-PCR. Only one isolate ZYMV-14P showed seed-borne transmission with transmission rate 2,6 %. This is the first detected seed-transmitted isolate in Ukraine. Phylogenetic analysis defined ZYMV-14P isolate as member of group A. This isolate was clustered with other known Ukrainian isolates and isolates from Hungary, Czech Republic, Austria and France within subgroup AI.

Key words: ZYMV, seed transmission, phylogenetic analysis.

Introduction. Zucchini yellow mosaic virus (ZYMV) is a single-stranded positive-sense RNA virus of the family *Potyviridae* that can result in yellowing, severe leaf and fruit deformation, especially on plants from *Cucurbitaceae* family, that can reduce yields up to 94 % [3]. Since its discovery this virus has spread rapidly and achieved the global distribution within two decades of its discovery [4].

ZYMV remains one of the most widespread and destructive viruses affecting plants from *Cucurbitaceae* family in Ukraine as well as in other countries [1]. ZYMV strains belong to three diverse phylogenetic groups (A, B and C), which have different biological and molecular properties and distribution [2]. ZYMV isolates circulating in Ukraine belong to phylogenetic group A and highly related to strains and isolates from Hungary, Czech Republic, Austria and France from subgroup AI [9].

Broad distribution of ZYMV is a result of long-distance transmission via seeds [8]. Seed transmission of ZYMV is varying among different isolates and hosts [7]. In Ukraine seed-transmitted isolates weren't detected before. To test the possibility of seed transmission of Ukrainian ZYMV isolates and determine the contribution of seed transmission to the epidemiology of ZYMV in our country we used *C. pepo* seeds and measured seed transmission of ZYMV isolates by visual observation, ELISA and RT-PCR. Moreover, we conducted phylogenetic analysis of seed-transmitted isolate and compared it with previously detected ones to determine the phylogenetic group attribution. Understanding the epidemiology and evolution of ZYMV is therefore a key to controlling this devastating crop disease.

Material and Methods. Plant samples were collected from different regions of Ukraine and screened for the presence of viral antigens. The seeds of pumpkins and squashes (*Cucurbita pepo* L.) were dried, weighed and kept at 5°C. Seed transmission experiments were conducted in a greenhouse under strict sanitary conditions. The seedlings were visually observed and all plants showing any abnormality (mosaic symptoms, leaf deformation, etc.) were further collected and examined in ELISA and RT-PCR.

Double-antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) was conducted using commercial test-system of Loewe (Germany). Plant material was homogenized in 0.1M phosphate buffered saline

(PBS), pH 7.4, 1:2 (m/v). Plant components were removed by centrifugation at 5.000 g for 20 minutes at +4°C using centrifuge PC-6. The supernatant was taken for further ELISA. DAS-ELISA was performed according to the manufacturer's recommendations. The results were checked at the wavelength of 405/630 nm using microplate reader Termo Labsystems Opsis MR (USA) with software Dynex Revelation Quicklink.

Total RNA was extracted from plant samples using RNeasy Plant Mini kit (Qiagen, UK). The results were confirmed by electrophoresis of nucleic acids in 1.5 % agarose gel. The two-step reverse transcription reaction (RT-PCR) was accomplished using two specific primers complementary to the part of Nib-coat protein (Nib-CP) genes of ZYMV producing the amplicons with expected size of 600 bp [5]. PCR amplification was assessed by electrophoresis in a 1.5 % agarose gel in TBE buffer (89 mM TRIS borate and 2 mM EDTA, pH 8.3) and stained in ethidium bromide. The purified amplicons were sequenced using Applied Biosystems 3730x1 DNA Analyzer with Big Dye terminators, version 3.1 (Applied Biosystems, USA).

The aligned sequences of Ukrainian ZYMV isolates were compared with published sequences of ZYMV strains and isolates available in the GenBank database using NCBI/BLAST (<http://www.ncbi.nlm.nih.gov/>). The phylogenetic analysis was conducted using MEGA 6 software. A phylogenetic tree was constructed using the Neighbor-Joining method.

Results and discussion. Symptomatic plant samples were collected in different regions of Ukraine (Vinnytsia, Zaporizhzhia, Kyiv, Kirovohrad, Odesa, Poltava, Lviv, Ivano-Frankivsk, Cherkasy and Chernihiv). ZYMV-infected plants developed yellow mosaics, leaf blade deformation, knobs and malformations of fruits (Fig. 1). DAS-ELISA was performed for detection of viral antigens in plant samples. 21 % of analyzed samples were infected by ZYMV.

All samples were also tested for the presence of *Cucumber mosaic virus* (CMV, family *Bromoviridae*), since mixed infections of CMV and ZYMV are common on cucurbitaceous plants and both viruses are seed-transmitted. The incidence of mixed infection among diseased plants was 5 %.

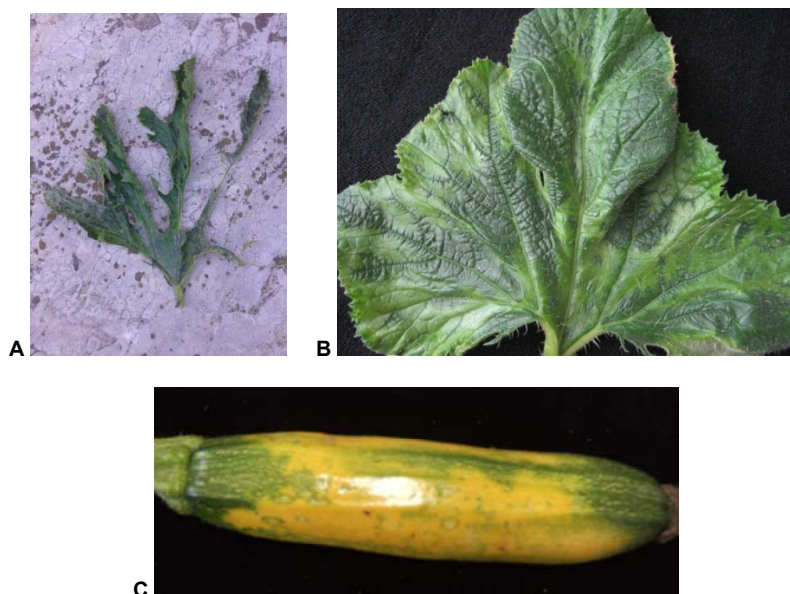


Fig. 1. Symptoms caused by ZYMV in field conditions: leaf deformation and dark green mosaic on squash (A), dark green mottling on pumpkin (B) and yellowing on squash (C)

Further, seeds from ZYMV-monoinfected plants were obtained and planted. In four weeks we observed typical symptoms of ZYMV: filamentary deformation and mosaics of leaf blades (Fig. 2). Symptomatic seedlings were marked

and analyzed. The rate was assessed by ELISA and RT-PCR. Only one isolate ZYMV-14P showed seed-borne transmission with transmission rate 2,6 %. This is the first detected seed-transmitted isolate in Ukraine.

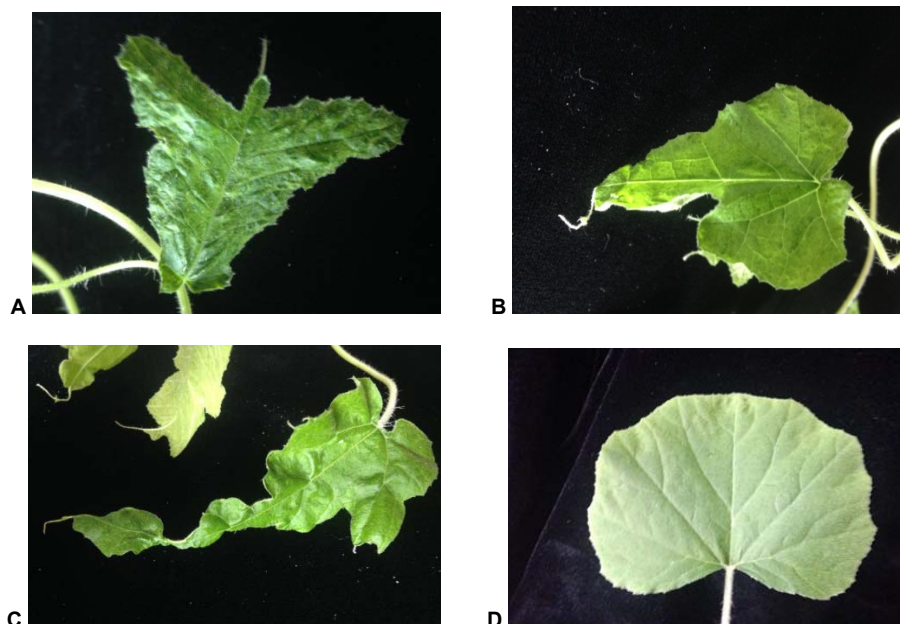


Fig. 2. Symptoms on pumpkin cultivated from infected seeds: mottling on leaf (A); deformation of leaf blade (B–C); control (D)

To investigate the genetic diversity among ZYMV-14P and previously characterized ZYMV isolates from Europe and other parts of the world the phylogenetic analysis was carried out. Nucleotide sequence of Nib-CP region of Ukrainian seed-transmitted isolate was obtained. The sequence data have been submitted to NCBI, accession

number – KY99102. Nib-CP-based phylogenetic tree was built based on nucleotide sequences of Ukrainian isolates and strains of different groups from Genbank (table) using Neighbor-Joining method in MEGA6. *Bean common mosaic virus* was used as outgroup.

Table. Strains and isolates of Zucchini yellow mosaic virus used for phylogenetic analysis in this work

Strain / isolate	Host (if mentioned)	Accession Number	Country	Nucleotide identity with ZYMV-14P, %
ZYMV-10G	<i>Cucurbita pepo</i>	KY99097	Ukraine	99,8
ZYMV-10P	<i>Cucurbita pepo</i>	KY99098	Ukraine	99,8
ZYMV-3814	<i>Citrullus lantanus</i>	KY99099	Ukraine	99,8
ZYMV-513	<i>Cucurbita pepo</i>	KY99100	Ukraine	100
ZYMV-B	<i>Cucumis melo</i>	KY99101	Ukraine	100
ZYMV-23	<i>Cucurbita pepo</i>		Ukraine	100
ZYMV-7	<i>Citrullus lantanus</i>		Ukraine	100
ZYMV-21	Zucchini		Ukraine	100
ZYMV-34	<i>Cucurbita pepo</i>		Ukraine	99,6
ZYMV-H	<i>Cucurbita pepo</i>	KF976712	Czech Republic	100
ZYMV-Kuchyna	<i>Cucurbita pepo</i>	DQ124239	Slovakia	99,5
ZYMV-Cg11-396	Zucchini	KJ815024	France	99,5
ZYMV-VE10-263	<i>Cucumis dipsaceus</i>	JX310107	Venezuela	96,2
ZYMV-Iraq	Zucchini	JQ026020	Iraq	96,7
ZYMV-Cu	<i>Cucumis sativus</i>	EU561043	Poland	96,7
ZYMV-P	Pumpkin (<i>Cucurbita moschata</i>)	AJ316227	China	95
ZYMV-Pak	<i>Lagereria siceneria</i>	AB127936	Pakistan	96,2
ZYMV-SYZ102	Squash	GU903893	Syria	97,3
ZYMV-NAT		EF062582	Israel	96,7
ZYMV-CI09-09	<i>Cucumis sativus</i>	CI09-09	Cote d'Ivoire	96,7
ZYMV-E9	Zucchini	HM641798	France	96,7
ZYMV-begonia	<i>Begonia</i>	AM422386	Taiwan	93,8
ZYMV-Cg11-379	Zucchini	KJ815022	France	100
ZYMV-Zuy	Zucchini	EU561044	Poland	90,9
ZYMV-Zug	Zucchini	EU561045	Poland	89,1
ZYMV-Florida		ZYMCP	USA	90,4
ZYMV-C-16		DQ645729	Spain	73,7
ZYMV-WM	<i>Citrullus lantanus</i>	AJ515911	China	89
ZYMV-KR-PA	<i>Cucurbita moschata</i>	AY278998	Korea	89,7
ZYMV-Cg10-253	Zucchini	KJ815014	France	82,5
ZYMV-RDA	<i>Cucurbita pepo</i>	AB369279	South Korea	70,2
ZYMV-Singapore		AF014811	Singapore	65
ZYMV-VN/Cm1	<i>Cucurbita moschata</i>	DQ925448	Vietnam	67,8
ZYMV-LG1	<i>Cucurbita pepo</i> var. <i>melo</i> (<i>zucchini</i>)	AJ889243	China	66,1
ZYMV-TY	<i>Thladiantha grosvenorii</i>	AJ889244	China	73
ZYMV-Knx-1	<i>Cucurbita moschata</i>	JF792363	Australia	73
ZYMV-Knx-3	<i>Cucumis melo</i>	JF792365	Australia	89,7
ZYMV-CH99/193	Squash	AY611023	China	89,7
ZYMV-WG	<i>Benincasa hispida</i>	AJ316229	China	92,2
ZYMV-TV1	<i>Cucurbita moschata</i>	KJ789918	Republic of South Africa	100
ZYMV-390-10	<i>Cucurbita pepo</i> 'Horgos'	JX262127	Serbia	98,9
ZYMV-670-40	<i>Cucurbita maxima</i>	JX262134	Serbia	97,3
ZYMV-SYR-B2	<i>Cucurbita pepo</i>	EU999757	Syria	100
ZYMV-Austria 12		AJ420017	Austria	73,2

Ukrainian seed-transmitted isolate ZYMV-14P was grouped with other previously detected Ukrainian isolates within subgroup A (Fig. 3). The members of this group are widespread all over the world. Ukrainian isolates were clus-

tered with isolates from Hungary, Czech Republic, Austria and France within subgroup AI. Obtained isolates were characterized with high homology (98–100 %).

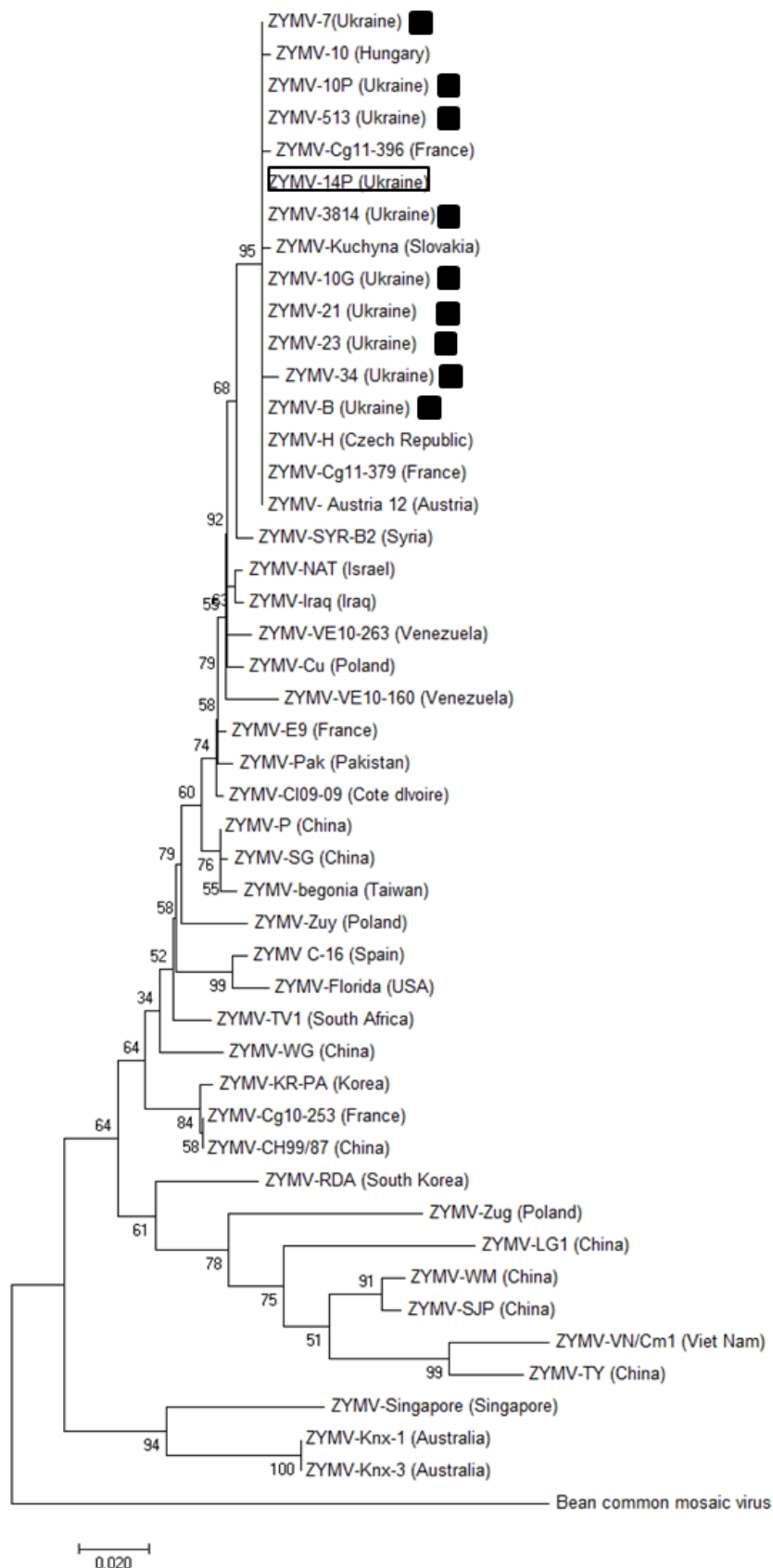


Fig. 3. Phylogenetic tree based on Nib-CP gene sequences of selected strains and Ukrainian isolates.

Bootstrap values are shown above branches.

Ukrainian isolates detected previously are marked by squares and seed-transmitted Ukrainian isolate – by rectangular box

Isolate ZYMV-14P belong to the same group that other known Ukrainian isolates and share high nucleotide identity of Nib-CP gene region with them (>99 %).

Conclusions. For the first time in Ukraine seed-transmitted ZYMV isolate was detected. Phylogenetic analysis shown this isolate is member of the most frequent phy-

logenetic group in the world. This group is widespread in other European countries.

The rate of seed transmission of ZYMV-14P is 2,6 %. According to literature data, average rate of ZYMV seed transmission is ranged from 0 to 18,9 % [2, 3]. This difference may be explained by different approaches to setting experiment (choosing model plant, method of virus detection (only visual or visual and ELISA/RT-PCR)).

Anyway, obtained data show us that careful testing and certification of cucurbit seeds should be compulsory for all producers and sellers in Ukraine. Absence of any control of viral infections is still the key obstacle for the prevention of dissemination of virus [6].

Список використаних джерел:

1. Cantliffe D. J. Current trends in cucurbit production in the U.S. / D. J. Cantliffe, N. L. Shaw, P. J. Stoffella // Acta. Hortic. – 2007. – Vol. 731. – P. 473–478.
2. Coutts B. A. Zucchini yellow mosaic virus: biological properties, detection procedures and comparison of coat protein gene sequences / B. A. Coutts // Archives of Virology. – 2011. – Vol. 156. – P. 2119–2131.
3. Coutts B. A. Minimising losses caused by Zucchini yellow mosaic virus in vegetable cucurbit crops in tropical, sub-tropical and Mediterranean environments through cultural methods and host resistance / B. A. Coutts, M. A. Kehoe, R. A. C. Jones // Virus Res. – 2011. – Vol. 159. – P. 141–161.
4. Desbiez C. Zucchini yellow mosaic virus / C. Desbiez, H. Lecoq // Plant Pathol. – 1997. – Vol. 46. – P. 809–829.
5. Desbiez C. Biological and serological variability, evolution and molecular epidemiology of Zucchini yellow mosaic virus (ZYMV, Potyvirus) with special reference to the Caribbean islands / C. Desbiez, C. Wipf-Scheibel, H. Lecoq // Virus Res. – 2002. – Vol. 85. – P. 5–16.
6. Munkvold G. P. Seed pathology progress in academia and industry / G. P. Munkvold // Annu. Rev. Phytopathol. – 2009. – Vol. 47. – P. 285–311.
7. Simmons H. E. Experimental verification of seed transmission of Zucchini yellow mosaic virus / H. E. Simmons // Plant Disease. – 2011. – Vol. 95. – P. 751–754.

8. Tóbiás I. Characterization of Hungarian isolates of zucchini yellow mosaic virus (ZYMV, potyvirus) transmitted by seeds of Cucurbita pepo var Styriaca / I. Tóbiás, L. Palkovics // Pest Manag Sci. – 2003. – Vol. 59, № 4. – P. 493–497.

9. Viruses infecting cucurbits in Ukraine and their phylogenetic analysis / O. Tymchyshyn, T. Shevchenko, I. Budzanivska, V. Polishchuk // Building bridges between disciplines for sustainable management of plant diseases. 13th International Plant Virus Epidemiology Symposium, June 6–10, 2016 : Abstracts. – Avignon, France, 2016. – P. 148.

References:

1. Cantliffe D., Shaw N., Stoffella P. Current trends in cucurbit production in the U.S. Acta. Hortic. 2007;731:473-8.
2. Coutts B. Zucchini yellow mosaic virus: biological properties, detection procedures and comparison of coat protein gene sequences. Archives of Virology. 2011;156:2119-31.
3. Coutts B., Kehoe M., Jones R. Minimising losses caused by Zucchini yellow mosaic virus in vegetable cucurbit crops in tropical, sub-tropical and Mediterranean environments through cultural methods and host resistance. Virus Res. 2011;159:141-61.
4. Desbiez C., Lecoq H. Zucchini yellow mosaic virus. Plant Pathol. 1997;46:809-29.
5. Desbiez C., Wipf-Scheibel C., Lecoq H. Biological and serological variability, evolution and molecular epidemiology of Zucchini yellow mosaic virus (ZYMV, Potyvirus) with special reference to the Caribbean islands. Virus Res. 2002; 85:5-16.
6. Munkvold G. Seed pathology progress in academia and industry. Annu. Rev. Phytopathol. 2009;47: 285-11.
7. Simmons H. Experimental verification of seed transmission of Zucchini yellow mosaic virus. Plant Disease. 2011;95:751-4.
8. Tóbiás I., Palkovics L. Characterization of Hungarian isolates of zucchini yellow mosaic virus (ZYMV, potyvirus) transmitted by seeds of Cucurbita pepo var Styriaca. Pest Manag Sci. 2003;59(4):493-7.
9. Tymchyshyn O., Shevchenko T., Budzanivska I., Polishchuk V. Viruses infecting cucurbits in Ukraine and their phylogenetic analysis. Proceedings of the 13th International Plant Virus Epidemiology Symposium, 2016 June 6-10, Avignon, France, 2016 p.148.

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ФІЛОГЕНЕТИЧНИЙ АНАЛІЗ УКРАЇНСЬКОГО ІЗОЛЯТУ ZUCCHINI YELLOW MOSAIC VIRUS, ЩО ПЕРЕДАЄТЬСЯ НАСІННЯМ

Zucchini yellow mosaic virus (ZYMV) є одним із найпоширеніших та шкодочинних вірусів, що інфікують рослини родини Cucurbitaceae в Україні та інших країнах. ZYMV на ранніх стадіях розвитку рослин може спричиняти значні втрати врожаю. Стаття присвячена дослідженню передачі українських ізолятів ZYMV насінням рослин Cucurbita pepo у лабораторних умовах за відсутності комах. Частота передачі вірусу насінням оцінювалась за допомогою ELISA та RT-PCR. Тільки один ізолят вірусу ZYMV-14P проявив здатність до насінневої передачі з частотою 2,6 %. Це перший український ізолят ZYMV, який достовірно передається насінням. За допомогою філогенетичного аналізу встановлено, що ZYMV-14P належить до групи А. Цей ізолят входить до одного кластера з іншими українськими ізолятами та ізолятами з Угорщини, Чехії, Австрії та Франції, які належать до підгрупи AI.

Ключові слова: ZYMV, передача насінням, філогенетичний аналіз.

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ФИЛОГЕНЕТИЧЕСКИЙ АНАЛИЗ УКРАИНСКОГО ИЗОЛЯТА ZUCCHINI YELLOW MOSAIC VIRUS, ПЕРЕДАЮЩЕГОСЯ СЕМЕНАМИ

Zucchini yellow mosaic virus (ZYMV) – один из широко распространенных и вредоносных вирусов, инфицирующих растения семейства Cucurbitaceae в Украине и других странах. ZYMV на ранних стадиях развития растений вызывает значительные потери урожая. Статья посвящена исследованию возможности передачи с помощью семян растений Cucurbita pepo украинских изолятов ZYMV в лабораторных условиях при отсутствии насекомых. Частота семенной передачи вируса оценивалась с использованием ELISA и RT-PCR. Только один изолят вируса ZYMV-14P проявил способность к семенной передаче с частотой 2,6 %. Это первый украинский изолят ZYMV, который достоверно передается семенами. При помощи филогенетического анализа установлено, что изолят ZYMV-14P входит в группу А подгруппы AI и формирует кластер с другими украинскими изолятами и изолятами из Венгрии, Чешской республики, Австрии и Франции.

Ключевые слова: ZYMV, передача семенами, филогенетический анализ.