In this work the peculiarities of reading comprehension from electronic, audio devices and hard copies were studied through comparative analysis of the learning accuracy and electrical activity of the brain when reading or listening to the text. Eighty students took part in the research. They were offered 2 passages of text from fiction and popular-scientific literature for reading, presented in a form of an e-book, MP3-format and in a printed copy. The level of comprehension and assimilation of the read material was checked by testing based on the content of the text immediately after reading and in 2 weeks. The comparative EEG analysis did not reveal significant differences in the spectral power of the studied ranges when reading a paper book and e-book. Differences were found when listening to audiobooks comparatively to reading. In general, the effectiveness of text learning does not depend on the way of its presentation, however, sex and individual traits of a person, such as preferred learning style and extraversion level, are more important.

Key words: reading, learning, paper books, electronic books, EEG, sex differences, extraversion.

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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 Loeve (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 Opis 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 (Vinnystia, Zaporizhzhia, Kyiv, Kirovohrad, Odesa, Poltava, Lviv, Ivano-Frankivsk, Chernsky 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%.

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.

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

**Figure 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).**

**Figure 2. Symptoms on pumpkin cultivated from infected seeds: mottling on leaves (A); deformation of leaf blade (A-C); control (D).**
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Number – KY99102. Nib-CP-based phylogenetic tree was built based on nucleotide sequences of Ukrainian isolates and strains of different groups from Genbank (Table 1) using Neighbor-Joining method in MEGA6. *Bean common mosaic virus* was used as outgroup.

**Table 1.** 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         | Cucurbita pepo      | KY99097          | Ukraine       | 99,8                                |
| ZYMV-10P         | Cucurbita pepo      | KY99098          | Ukraine       | 99,8                                |
| ZYMV-3814        | Citrullus lantanus  | KY99099          | Ukraine       | 99,8                                |
| ZYMV-513         | Cucurbita pepo      | KY99100          | Ukraine       | 100                                 |
| ZYMV-B           | Cucumis melo        | KY99101          | Ukraine       | 100                                 |
| ZYMV-23          | Cucurbita pepo      |                  | Ukraine       | 100                                 |
| ZYMV-7           | Citrullus lantanus  |                  | Ukraine       | 100                                 |
| ZYMV-21          | Zucchini            |                  | Ukraine       | 100                                 |
| ZYMV-34          | Cucurbita pepo      |                  | Ukraine       | 99,6                                |
| ZYMV-H           | Cucurbita pepo      | KF976712         | Czech Republic| 100                                 |
| ZYMV-Kuchyna     | Cucurbita pepo      | DQ124239         | Slovakia      | 99,5                                |
| ZYMV-Cq11-396    | Zucchini            | KJ815024         | France        | 99,5                                |
| ZYMV-VE10-263    | Cucumis dipaceae    | JX310107         | Venezuela     | 96,2                                |
| ZYMV-Iraq        | Zucchini            | JQ026020         | Iraq          | 96,7                                |
| ZYMV-Cu          | Cucumis sativus     | EU561043         | Poland        | 96,7                                |
| ZYMV-P           | Pumpkin (Cucurbita moschata) | AJ316227 | China       | 95                                 |
| ZYMV-Pak         | Lageneria siceneria | AB127936         | Pakistan      | 96,2                                |
| ZYMV-SZY102      | Squash              | GU903893         | Syria         | 97,3                                |
| ZYMV-NAT         |                     | EF082582         | Israel        | 96,7                                |
| ZYMV-C109-09     | Cucumis sativus     | CI09-09          | Cote d’Ivoire| 96,7                                |
| ZYMV-E9          | Zucchini            | HM041798         | France        | 96,7                                |
| ZYMV-begonia     | Begonia             | AM422356         | Taiwan        | 93,8                                |
| ZYMV-Cq11-379    | Zucchini            | KJ815022         | France        | 100                                 |
| ZYMV-Zuy         | Zucchini            | EU561044         | Poland        | 90,9                                |
| ZYMV-Zug         | Zucchini            | EU561045         | Poland        | 89,1                                |
| ZYMV-Florida      |                     |                  | USA           | 90,4                                |
| ZYMV-C-16        |                     | DQ645729         | Spain         | 73,7                                |
| ZYMV-WM          | Citrullus lantanus  | AJ515911         | China         | 89                                 |
| ZYMV-KR-PA       | Cucurbita moschata  | AJ278998         | Korea         | 89,7                                |
| ZYMV-Cg10-253    | Zucchini            | KJ815014         | France        | 82,5                                |
| ZYMV-RDA         | Cucurbita pepo      | AB369279         | South Korea   | 70,2                                |
| ZYMV-Singapore   |                     | AF014811         | Singapore     | 65                                 |
| ZYMV-VN/Cm1      | Cucurbita moschata  | DQ825448         | Vietnam       | 67,8                                |
| ZYMV-LG1         | Cucurbita pepo var. melopepo (zucchini) | AJ898243 | China       | 66,1                                |
| ZYMV-TY          | Thladiantha grosvenorii | AJ898244 | China       | 73                                 |
| ZYMV-Knx-1       | Cucurbita moschata  | JF792363         | Australia     | 73                                 |
| ZYMV-Knx-3       | Cucumis melo        | JF792365         | Australia     | 89,7                                |
| ZYMV-CH99/193    | Squash              | AY611023         | China         | 89,7                                |
| ZYMV-WG          | Banincasa hispida   | AJ316229         | China         | 92,2                                |
| ZYMV-TV1         | Cucurbita moschata  | KJ899918         | Republic of South Africa | 100 |
| ZYMV-390-10      | Cucurbita pepo 'Horgos' | JX262127 | Serbia   | 98,9                                |
| ZYMV-670-40      | Cucurbita maxima    | JX262134         | Serbia        | 97,3                                |
| ZYMV-SYR-B2      | Cucurbita pepo      | 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 clustered with isolates from Hungary, Czech Republic, Austria and France within subgroup AI. Obtained isolates were characterized with high homology (98-100%).
Figure 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, the 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].

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