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PHYLOGENY OF *PLECTOSPHAERELLA MELONIS* STRAIN 502 AND VARIETAL SENSITIVITY OF CUCUMBER PLANTS

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Aim. To investigate the phylogenetic relations of *P. melonis* strain 502 and to study the varietal sensitivity of cucumber plants to *P. melonis* strain 502. **Methods.** DNA was extracted using the enzymatic lysis buffer. The PCR was conducted following White et al. protocol (1990). The obtained PCR-products were determined by sequencing on the automatic capillary sequencer Applied Biosystems ABI Prism 3130. The sequence of the gene 5.8S rRNA of *P. melonis* strain 502 was compared to the sequences from the GenBank database using the BLAST analysis. The phylogenetic analysis was conducted by the neighbor-joining method. The evolutionary distances were estimated by the method of Jukes & Cantor. The evolutionary analysis was conducted in MEGA7. The sensitivity of cucumber plants was determined during a vegetative experiment with artificial infection background (AIB), created by introducing the infectious material of fungus *P. melonis* strain 502 into the soil. The infectious material was introduced at a rate of 50 thousand CFU/per 1 g of soil. The damage to the root system was assessed after 14 days of cultivating plants on the AIB. The disease severity index (DSI) was estimated to determine the general sensitivity of the investigated varieties. The varieties, which received DSI <2.0, were considered highly resistant, from 2.0 to 2.9 – moderately resistant, from 3.0 to 3.9 – susceptible, and 4.0 or higher – very susceptible. The statistical methods and Statistica 12 (Stat-Soft Inc., USA) were used to assess the reliability of the experimental data. The arithmetic mean and the square deviation (SD) were calculated to assess the DSI at $p < 0.05$. **Results**. The PCR method was used to obtain a sequence of *P. melonis* strain 502 of 317 bp and to conduct the phylogenetic analysis. The search of BLASTn in GenBank showed that the sequence of ITS *P. melonis* strain 502 had 99 % homology to 10 isolates of *P. melonis*. California isolate CA-1103 differed from *P. melonis* strain 502 only by one pair of nucleotides. The homology percentage was also 99 % to the isolates from Texas (TX-1060 and TX-1065), Japan (CBS 489.96 and ACCC:39138), and Italy (Plect 211 and Plect 212). Spanish isolates (A-943, A-537, A-99) had a smaller percentage of homology – 98 %. The distribution of the disease for varieties Zhuravlionok, Konkurent, and Rodnichok was 50 %, and for varieties Nizhynsky 12 and Liosha – 60 and 80 %, respectively. The study on the varietal sensitivity to *P. melonis* strain 502 demonstrated that varieties Konkurent (DSI – 1.3 ± 0.0) and Rodnichok (DSI – 1.5 ± 0.1) were referred to the group of highly resistant ones, Zhuravlionok (DSI – 2.6 ± 0.3), Liosha (DSI – 2.2 ± 0.2) and Nizhynskyi 12 (DSI – 2.5 ± 0.2) – moderately resistant. The root collar was most severely diseased; it was of brown color. There were no above-ground symptoms. Additional roots above the damaged area were observed in cucumber plants of Zhuravlionok, Konkurent, and Rodnichok. **Conclusions**. The phylogenetic analysis demonstrated that *P. melonis* strain 502 had 99 % homology with 10 isolates of *P. melonis* from the USA, Japan, and Italy. The percentage of homology with Spanish isolates, first isolated as the representatives of this genus, was lower (98 %). The root collar was damaged the most, which also indicated the similarity of symptoms to the American isolates compared to the ones from Spain, where the hypocotyl was damaged. There were no above-ground disease symptoms. The distribution of the disease and the degree of the damage were different depending on the variety. They did not depend on the maturity group, which demonstrated individual varietal sensitivity and the relevance of selecting resistant varieties as one of the main ways to control this disease agent. The study of the varietal sensitivity of *C. sativus* to *P. melonis* strain 502 showed that varieties Konkurent and Rodnichok could be referred to the highly resistant group, while Zhuravlionok, Liosha, and Nizhynskyi 12 – to moderately resistant ones. The distribution of the disease was also different depending on the variety (Zhuravlionok, Konkurent, and Rodnichok – 50 %, Nizhynsky 12 and Liosha – 60 and 80 %, respectively). Additional roots above the damaged area were observed in cucumber plants of varieties Zhuravlionok, Konkurent, and Rodnichok as a defensive response to being infected by

the pathogen. No response of oversensitivity was registered in the investigated varieties of cucumber plants regarding *P. melonis* strain 502, which was conditioned by the stability of the environmental factors and absence of abiotic stressors for the plant, including moisture shortage and high temperatures.

Key words: *Plectosphaerella melonis*, *Cucumis sativus*, soilborne disease, pathogen, phylogenetic analysis.

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INTRODUCTION

In recent years, a new disease of Cucurbitaceae plants, caused by such disease agent as *Plectosphaerella melonis* (syn. *Acremonium cucurbitacearum*, *Nodulisporium melonis*) was registered in many countries (Garsia-Jimenez et al, 1994; Bruton et al, 1995; Gubler et al, 1996; Alfaro-Garsia et al, 1996; Armengol, 1997; Armengol et al, 1998; Bruton et al, 1999; Bruton et al, 2000a, b; Aergeten et al, 2000; Martinez-Culebras et al, 2004; Chilosi et al, 2008). The disease was first registered in Spain after the massive collapse of melons at the stage of large-scale fruit-bearing (Garsia-Jimenez et al, 1994; Alfaro-García et al, 1996). In the USA, Bruton et al (1995) reported the collapse of watermelons and muskmelons in the state of California, which became the foundation for further studies of California isolates of *P. melonis* (Aegerter et al, 2000). In 1996, Bruton et al. reported this disease in melon plants in the state of Texas. In 2002, *P. melonis* was also isolated from diseased melon plants in Italy (Chilosi et al, 2008). Previously, there were reports about the disease cases on Cucurbitaceae plants, but it is known now that the fungus infects other families as well. For instance, it was the infectious agent for basil, parsley, tomatoes, and sweet pepper (Carlucci et al, 2012; Raimondo and Carlucci 2018 a, b).

In Ukraine, this disease was first registered in 2012 in cucumber plants, cultivated in the covered soil, where *P. melonis* strain 502 was isolated (Kopilov et al, 2021). We have already studied the capability of the cultural liquid of *P. melonis* 502 to regulate the growth of plants depending on the test culture and to synthesize the phytohormone ethylene *in vitro* (Tsekhmister et al, 2021); we have also investigated the histotrophic localization of the pathogen and its ability to synthesize cellulase enzymes depending on environmental conditions (Patyka et al, 2022), and in this study, our work aimed to demonstrate the phylogenetic relations between *P. melonis* strain 502 and the isolates from other countries and to study the sensitivity of different varieties of *Cucumis sativus* L.

MATERIALS AND METHODS

Strain. A natural strain of fungus *P. melonis* strain 502, isolated from the diseased roots of cucumber plants (*C. sativus* cv Koroliok), cultivated in the covered soil, was used in the work (Kopilov et al, 2021; Tsekhmister et al, 2021). The strain was deposited in the Depository of the Institute of Microbiology and Virology, NAS of Ukraine, and numbered IMV F-100138. The sequence of the gene 5.8S rRNA of 317 bp was deposited in the GenBank database and numbered MK736305.1.

The genetic identification was conducted in the Department of Molecular Oncogenetics, the Institute of Molecular Biology and Genetics, NAAS (Kyiv, Ukraine) at the request of the Institute of Agricultural Microbiology and Agroindustrial Manufacture, NAAS (Chernihiv, Ukraine) within the scientific project No. 0116U003070.

The genomic DNA was extracted by preliminary lysis of the cellular walls of the fungus. To isolate the DNA sample, the fungus colony of *P. melonis* strain 502, cultivated on wort agar, was resuspended in 500 µl of the lysis buffer (guanidium thiocyanate – 49 %, Tris-HCl (pH 6.4) – 50 mM, EDTA (pH 8.0) – 20 mM, triton X-100 – 1 %). DNA fragments were absorbed by the DNA-sorbent "Silica". The mixture of DNA fragments and the sorbent was centrifuged for 1 min at 5,000 rpm, and the supernatant was removed. The remaining precipitate was added 300 µl of the washing solution (guanidium thiocyanate – 55 % and Tris-HCl (pH 6.4) – 50 mM), mixed, and centrifuged for 1 min at 5,000 rpm. The supernatant was removed. Then the precipitate was washed by adding 500 µl of the solution, containing 96 % ethanol – 80 %, Tris-HCl (pH 7.5) – 10 mM. It was mixed and centrifuged for 1 min at 10,000 rpm. The supernatant was removed, and the procedure was repeated once again. The remaining precipitate was dried for 5 min at 65 °C; then 100 µl of TE-buffer was added (Tris – 242.28 g, glacial acetic acid – 57 ml, 0.5 M EDTA (pH 8.0) – 100 ml, distilled water – 1,000 ml). The solution was mixed and kept in the thermostat at 65 °C for 5 min. After heating, the solution was mixed again and centrifuged for 15 min at 14,000 rpm. The obtained DNA solution was transferred to 0.5 ml tubes and kept at –20 °C.

PCR analysis. The ITS site (ITS1, 5.8S rDNA and ITS2) was used for PCR with primers ITS1 and ITS4

(White et al, 1990). The amplification reaction was conducted using Applied Biosystems equipment with the working solutions of primers (10 µl ITS1, 10.4 µl ITS4, and 29.6 µl of deionized water). The PCR reaction mixture contained 5 µl PCR-buffer, 2.5 µl dNTP, 1 µl of the mixture of primers, 0.2 µl Taq DNA-polymerase, and 1 µl of the DNA sample. The PCR was conducted for 35 cycles (94 °C – 20 s, 55 °C – 20 s, 72 °C – 30 s). Thermo Scientific MassRuler Low Range DNA Ladder Ready-to-Use 80-1031 was used for quantification and sizing of PCR amplicons. The obtained PCR products were sequenced on the automatic capillary sequencer Applied Biosystems ABI Prism 3130.

The sequence of the gene 5.8S rRNA of *P. melonis* strain 502 was compared to the sequences from the GenBank database using the BLAST analysis http://www.ncbi.nlm.nih.gov/blast).

Phylogenetic analysis, alignment of nucleotide sequences. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances are computed using the Jukes-Cantor method (Jukes and Cantor, 1969) and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. The evolutionary analyses were conducted in MEGA7 (Kumar et al, 2016).

The sensitivity of cucumber plants of varieties Liosha, Zhuravlionok, Konkurent, and Rodnichok against *P. melonis* strain 502 was determined during the pot experiment. The plants were grown for 28 days on soil artificially infested with the *P. melonis* strain 502 at a rate of 50 thousand CFU/1 g of soil. 10 seeds per pot were sown at a depth of 2–3 cm. After the germination, the plants were reduced to 7 plants per pot. The experiment was done in three repeats. The cucumber plants, grown on soil without inoculum, served as control. The pots with plants were placed in the greenhouse at random under natural light. The soil humidity was kept at 60 % of the full moisture capacity. The damage to the root system was assessed after 14 days of cultivating plants, according to Bruton et al (2000b). The distribution of the disease was determined as the percentage of damaged plants from the total number of plants under investigation. The disease severity index (DSI) was estimated to determine the general sensitivity of the varieties under investigation according to the method, developed for this disease (Bruton et al, 2000b). The varieties, which received DSI < 2.0, were considered highly resistant, from 2.0 to 2.9 – moderately resistant, from 3.0 to 3.9 – susceptible, and 4.0 or higher – very susceptible.

The inoculum preparation was made according to the methods of (Tkachik, 2014). The fungus culture of *P. melonis* strain 502 was reproduced in 1 l flasks on a solid substrate, containing oats seeds, oats flakes, water, chalk, and gypsum. The substrate was sterilized twice at 128 °C and a pressure of 1.5 atmospheres for 1 h 30 min. The inoculum was a pure culture of *P. melonis* strain 502, isolated onto a solid culture medium of wort agar from the diseased cucumber plants. The sowing material was obtained by washing conidia and fragments of fungal hyphae from oblique wort agar. The flasks were kept in the thermostat at 26 ± 2°C. After the substrate was covered with fungus mycelium (Day 21), it was transferred to paper bags, dried to a stable mass at 30 °C, and ground. To determine the titer of the infectious material, Gorjaev's chamber was used; the sowing was done on a solid culture medium of wort agar (4–5 % of dry substances).

Statistical methods. The experiment had three repeats. The statistical methods and Statistica 12 (StatSoft Inc., USA) were used to assess the reliability of the experimental data. The arithmetic mean and the square deviation (SD) were calculated to assess the DSI at the value level of $p < 0.05$.

RESULTS

The Nucleotide BLAST search in GenBank showed that the sequence of ITS *P. melonis* 502 (MK736305.1) had 99 % homology to 10 isolates of *P. melonis* (Table 1, Fig. 1). Fig. 1 demonstrates the results of the phylogenetic analysis of the obtained sequence with sequences of reference strains of *Plectosphaerella* fungi from the GenBank database. The representatives of *Acremonium* genus were accepted as an outgroup. California isolate CA-1103 differed from *P. melonis* strain 502 only by one pair of nucleotides. The homology percentage was also 99 % to the isolates from Texas (TX-1060 and TX-1065), Japan (CBS 489.96 and ACCC:39138), and Italy (Plect 211 and Plect 212). The isolates of *P. cucumerina* and *Acremonium* genus are on other branches, demonstrating their evolutionary remoteness from *P. melonis*. Almost all the isolates of *P. melonis* are in one branch except for the Iranian isolate CBS 411.95, located in another clade. Generally, the

Table 1. The source and number of adding ITS to GenBank, used for the phylogenetic analysis

Species	Strain	Number of adding to GenBank	Host plant	Source of extraction	Country	% of homology
Plectospaerella melonis (Acremonium cucurbitacearum, Nodulosporium melonis)	502	MK736305.1	*Cucumis sativus*	root	Chernihiv, Ukraine	–
A. cucurbitacearum	CA-1509	AJ621768.1	*Cucumis melo*	–	California, USA	–
A. cucurbitacearum	CA-1103	AJ621767.1	*C. melo*	–	California, USA	99.67
P. melonis	CBS:488.96	MH862586.1	–	–	Japan	–
P. melonis	CBS:489.96	MH862587.1	–	–	Japan	99.33
P. melonis	ACCC:39184	KY399814.1	*C. melo*	root	Texas, USA	–
P. melonis	ACCC:39185	KY399813.1	*Cucurbita melo*	root	Asaba-chou, Shizuok, Japan	99.33
P. melonis	228Plect	HQ238967.1	*C. melo*	–	Italy	–
P. melonis	Plect 212	HQ238966.1	*C. melo*	–	Italy	99.33
P. melonis	Plect 211	HQ238965.1	*C. melo*	–	Italy	99.33
A. cucurbitacerum	CBS 410.95	DQ825965.1	–	–	–	99.33
A. cucurbitacerum	CBS 408.95	DQ825963.1	–	–	–	99.33
N. melonis	CBS 488.96	AJ621769.1	*C. melo*	–	Japan	–
A. cucurbitacerum	TX-0092	AJ621765.1	*C. melo*	–	Texas, USA	–
A. cucurbitacerum	TX-1025	AJ621764.1	*C. melo*	–	Texas, USA	–
A. cucurbitacerum	TX-1065	AJ621761.1	*C. melo*	–	Texas, USA	99.33
A. cucurbitacerum	A-943	AJ621759.1	*C. melo*	–	Spain, Valencia	–
A. cucurbitacerum	A-537	AJ621758.1	*C. melo*	–	Spain, Valencia	–
A. cucurbitacerum	A-549	AJ621757.1	*C. melo*	–	Portugal	–
A. cucurbitacerum	A-548	AJ621756.1	*C. melo*	–	Portugal	–
A. cucurbitacerum	A-544	AJ621755.1	*Citrullus lanatus var. lanatus*	–	Spain, Canary Islands	–
A. cucurbitacerum	A-419	AJ621754.1	*C. melo*	–	Spain:Ciudad Real	–
A. cucurbitacerum	TX-1054	AJ621763.1	*C. melo*	–	USA, Texas	–
A. cucurbitacerum	TX-1060	AJ621762.1	*C. melo*	–	USA, Texas	99.00
A. cucurbitacerum	A-99	AJ621760.1	*C. melo*	–	Spain, Valencia	98.33
A. cucurbitacerum	CBS 409.95	DQ825964.1	–	–	Iran	–
P. melonis	Plect 148	HQ238968.1	*C. melo*	–	Italy	–
A. cucurbitacerum	CA-0133	AJ621766.1	*C. melo*	–	USA, California	–
A. cucurbitacerum	CBS 411.95	DQ825966.1	–	–	Iran	–
P. cucumerina	UASWS1827	MH673612.1	*tomato*	root	Switzerland	–
P. cucumerina	A1_T51	MH935031.1	*Acer pseudoplatanus*	symptomatic, surface-sterilized leaf	Italy	–
P. cucumerina	CBS:367.73	MH860704.1	–	–	Egypt	–
P. cucumerina	CBS:139.60	MH857926.1	–	–	USA	–
P. cucumerina	CBS:137.37	MH855856.1	–	–	Italy	–
P. cucumerina	CBS:355.36	MH855820.1	–	–	Netherlands	–
P. cucumerina	AS23	MG583754.1	–	–	–	–
P. cucumerina	P6215	MH063755.1	*Brassica napus*	surface-sterilized, asymptomatic roots	Germany	–

Continuation of Table 1

Species	Strain	Number of adding to GenBank	Host plant	Source of extraction	Country	% of homology
P. cucumerina	P6208	MH063585.1	*B. napus*	surface-sterilized, asymptomatic roots	France	–
P. cucumerina	SFC102149	MF186126.1	*Arctoscopus japonicus*	egg masses	South Korea	–
P. cucumerina	UT-Pl	MF161100.1	–	–	–	–
P. cucumerina	P01	MF033426.1	*Cucurbita pepo*	–	Taiwan, Fenglin	–
A. alcalophilum	JCM 7366	AB540579.1	–	Sludge from a compost made of pig feces	Japan	
A. crotocinigenum	cc56	DQ882846.1	–	rotting wood	Ecuador, Rio Palenque Forest Reserve	–
A. glaucum (Sarocladium glaucum)	CBS 796.69	NR_130686.1	–	–	–	–
A. strictum	1340	AM262390.1	*Dactylis glomerata*	–	Spain	–
A. hennebertii	CBS 768.69	MH859420.1	–	–	Zaire	–
A. sclerotigenum	CBS:124.42	LC144892.1	–	–	France	–

origin of *P. melonis* strain 502 remains unknown since the percentage of homology with American, Japanese, and Italian isolates is high. Spanish isolates (A-943, A-537, A-99), first isolated as representatives of this genus, have a smaller percentage of homology – 98 % and less.

The study on the varietal sensitivity (Table 2) to *P. melonis* strain 502 demonstrated the absence of hypocotyl damage in all the investigated varieties, while the root collar was very sensitive, in particular, in varieties Zhuravlionok (highly susceptible – 4.0) and Nizhynskyi 12 (susceptible – 3.0). The damage to the primary root and side roots were within the range of 1.4–2.6 and 1.4–3.3, respectively, and all the varieties, except for Nizhynskyi 12, were characterized by this feature as highly or moderately resistant. The side roots of Nizhynskyi 12 variety were referred to the susceptible group (3.3).

Fig. 2, *c* presents the diseased root system of cucumber plants of Liosha variety. In particular, the side roots were degraded, and the primary root was brown. The plants of Nizhynskyi 12 variety demonstrated a similar tendency.

Additional roots above the damaged area were observed in cucumber plants of varieties Zhuravlionok, Konkurent, and Rodnichok. The additional roots in varieties Liosha and Nizhynskyi 12 were either very scarce or completely absent. The pot experiment demonstrated that varieties Zhuravlionok, Konkurent, and Rodnichok enacted their mechanisms of defending against root pathogens and activated the process of root system regeneration faster as compared to varieties Liosha and Nizhynskyi 12.

It is noteworthy that even in case of severe damage to the root system, the above-ground symptoms of the disease were absent 14 days later. The cucumber plants of varieties Zhuravlionok, Konkurent, and Rodnichok, for which the activation of the root system regeneration was observed, were taller as compared to the control plants.

The cucumber plants of Liosha variety demonstrated a similar tendency 28 days later – they had a somewhat poorer root system and insignificant damage to the primary and side roots. The root collar was most severely diseased; it was of brown color (Fig. 2, *f*). There were no ground or above-ground symptoms.

The distribution of the disease for varieties Zhuravlionok, Konkurent, and Rodnichok was 50 %, for varieties Nizhynsky 12 and Liosha – 60 and 80 %, respectively (Table 2).

The study on the varietal sensitivity (Fig. 2, Table 2) to *P. melonis* strain 502 demonstrated that varieties Konkurent (DSI – 1.3 ± 0.0) and Rodnichok (DSI –

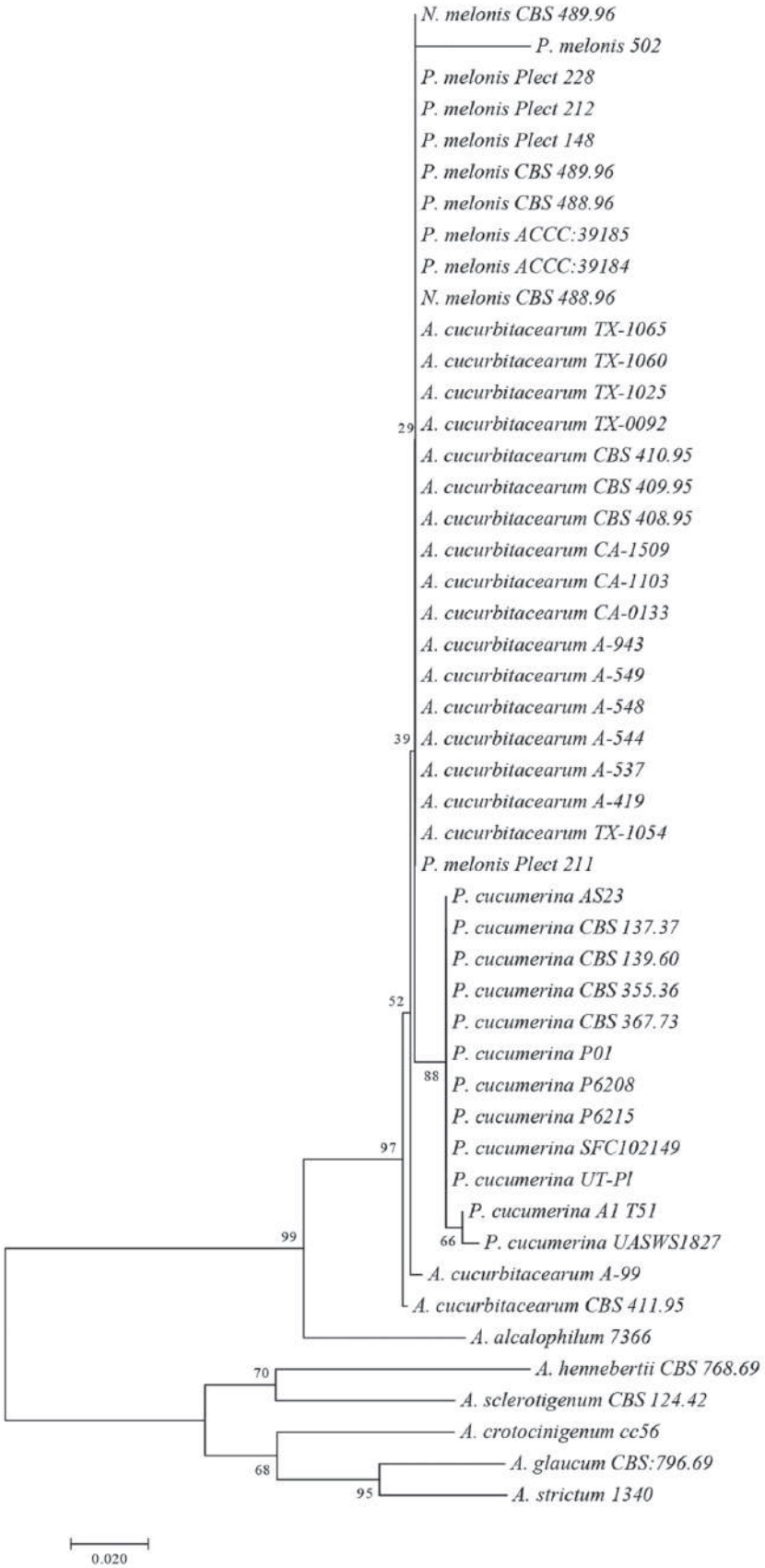

Fig. 1. The phylogenetic tree constructed by the neighbour-joining analysis of the partial 5.8S rRNA sequences of *P. melonis* strain 502 and references *P. melonis* from GenBank. The sequences of *Acremonium* genus fungi were accepted as an outgroup

Table 2. The damage to the root system of *C. sativus* after 14 days of the artificial inoculation with *P. melonis* strain 502

Variety	Maturity (days from germination to fruit-bearing)	DSI	Hypocotyl	Stem-root junction	Primary root	Secondary roots	Distribution of disease, %
Zhuravlionok (n = 8)	mid-early (40–45)	2.6 ± 0.3	1.0 ± 0.0	4.0 ± 0.0	2.6 ± 0.1	2.8 ± 0.1	50
Konkurent (n = 10)	mid-early (46–52)	1.3 ± 0.0	1.0 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	50
Rodnichok (n = 9)	mid-early (50–55)	1.5 ± 0.1	1.0 ± 0.0	1.6 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	50
Nizhynskyi 12 (n = 7)	middle-late (50–65)	2.5 ± 0.2	1.0 ± 0.0	3.0 ± 0.1	2.6 ± 0.1	3.3 ± 0.2	60
Liosha (n = 8)	early-season (36–38)	2.2 ± 0.2	1.0 ± 0.0	2.9 ± 0.1	2.1 ± 0.1	2.6 ± 0.2	80

Note. Rated 1 to 5, where 1 is healthy and 5 is severely diseased. Disease severity index (DSI) is the average of four individual root ratings. Mean ± one standard error.

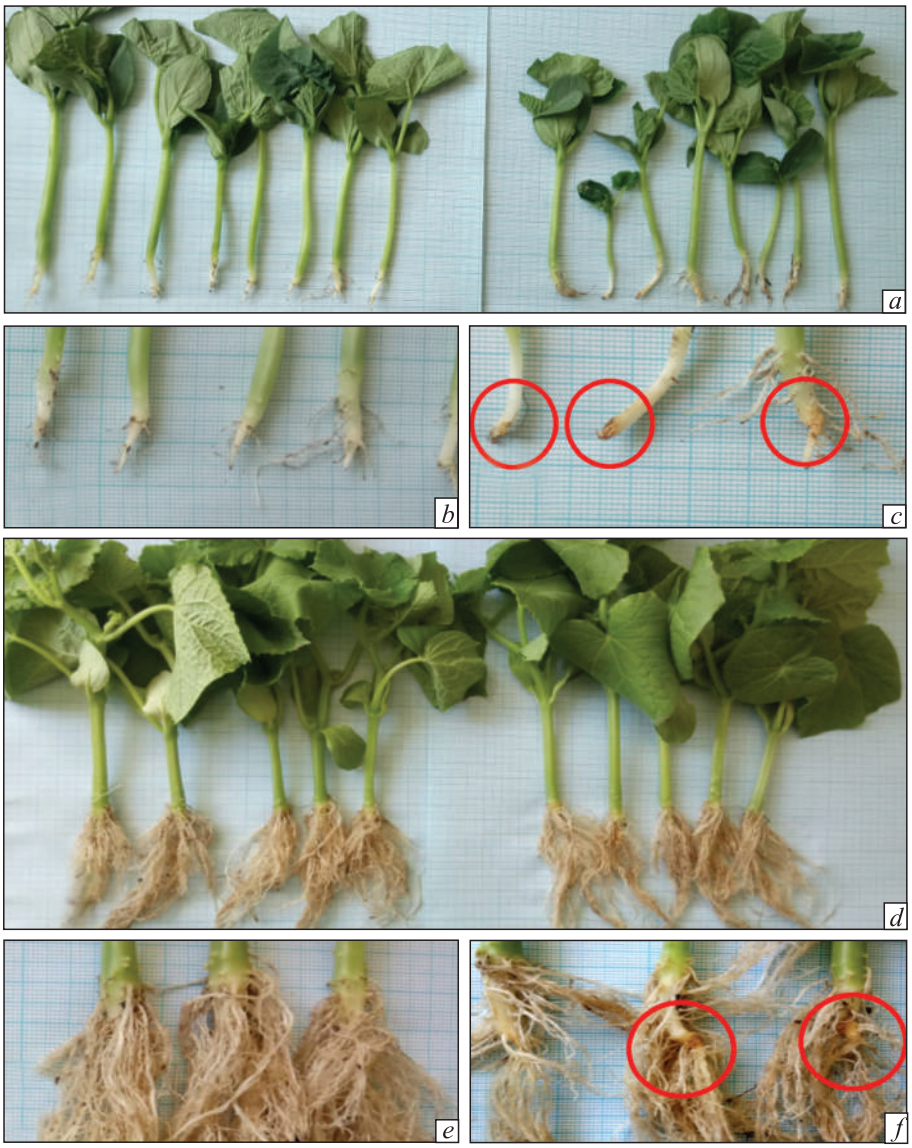

Fig. 2. Healthy (*a* – to the left, *b*) and diseased (*a* – to the right, *c*) cucumber plants of Liosha variety after two weeks since sowing seeds into the soil. Healthy (*d* – to the left, *e*) and diseased (*d* – to the right, *f*) cucumber plants of Liosha variety after four weeks since sowing seeds into the soil

1.5 ± 0.1) were referred to the group of highly resistant ones, Zhuravlionok (DSI – 2.6 ± 0.3), Liosha (DSI – 2.2 ± 0.2), and Nizhynskyi 12 (DSI – 2.5 ± 0.2) – to the moderately resistant ones.

DISCUSSION

The issue of the origin of *P. melonis* is yet to be answered, but the disease was found in North America and Europe within the same time period (Garsia-Jimenez et al, 1994; Bruton et al, 1995). The molecular and genetic studies and the analysis of vegetative affinity of the isolates demonstrated that the American isolates were similar or identical to the Spanish ones (Abad et al, 2000), and the cluster analysis based on RAPD models divided the isolates from Spain and the USA into two main groups (Martinez-Culebras et al, 2004). Our phylogenetic analysis of *P. melonis* strain 502 demonstrated a close affinity of 99 % to 10 isolates from the USA, Japan, Italy, and 98 % – to the Spanish isolate. It is well seen on the phylogenetic tree that the isolates of *P. melonis*, *A. cucurbitacearum* and *N. melonis* are in one clade, which is another confirmation of the synonymity of these species, which was shown by Carlucci et al (2012). It is also evident that the isolates of *P. cucumerina* are in another clade, and the isolates of *Acremonium* genus, taken as an outgroup, are quite distant. Similar results were previously shown by Martinez-Culebras et al (2004).

The phylogenetic studies of Spanish and American isolates demonstrate their close affinity, but the response of the plants to the effect of pathogens is different. Armengol and Bruton note that the decisive factor in the development of the pathological process is the environmental conditions, which differ in Spain and the USA (Bruton et al., 1999). Aegerter says that a relevant factor, affecting the development of symptoms, is heat stress (Aegerter et al., 2000). The morbific response of the plants to *P. melonis* differs depending on the cultivation conditions (covered or open soil), the interaction between the pathogen and competing microorganisms, and other ecological and trophic relations (Bruton et al, 2000b). For instance, the California isolates of *P. melonis* in field small plot experiments were found to be low pathogenic for melon, but under the greenhouse conditions, the degree of plant damage was much higher (Aegerter et al, 2000). Generally, a considerable impact of the environmental conditions and the uncontrolled nature of ecological factors on the development of the mentioned disease made its study rather complicated (Iglesias et al, 2000). In our study, no response of oversensitivity was registered in the investigated cucumber plants regarding *P. melonis* strain 502, which was conditioned by the stability of the environmental factors and absence of abiotic stressors for the plant, including moisture shortage and high temperatures. The way, in which the fungus will manifest itself, depends both on the physiological specificities of the plant and on the environmental conditions (Redman et al, 2001).

Our results of the varietal sensitivity study of *C. sativus* to *P. melonis* strain 502 are in agreement with the studies of American and Spanish isolates of *P. melonis*, which state that the pathogen brings the worst damage to plant roots in the initial stages of growth and development (Alfaro-Fernández and García-Luis, 2009; Biernaki and Bruton, 2001). Young sprouts have a low resistance to the infection, since some mechanisms, present in plants on later stages of development, are absent from their recently formed tissues (Alfaro-Fernández and García-Luis, 2009; Bruton et al, 2000b). Previously we have demonstrated that the plants of Nizhynskyi 12 variety are susceptible on the stage of two actual leaves (after two weeks of growing plants on the AIB of *P. melonis* 502) (Kopilov et al, 2021).

While studying the American isolates of *P. melonis*, Bruton et al also noted that only some parts of the root, namely the root collar, were the most susceptible (Bruton et al, 2000a,b), whereas J. Garsia-Jimenez et al (1994) investigated the Spanish isolates and found the worst damage in the hypocotyl. In our study, the worst damage was found in the root collar, and our results were in agreement with the study results for the American isolates of *P. melonis*.

The cucumber plants of varieties Zhuravlionok, Konkurent, and Rodnichok, for which the activation of the root system regeneration was observed, were taller as compared to the control plants. The improvement in the growth and development of these plants may be conditioned by the synthesis of growth-regulating compounds, stimulating the ontogenesis processes in the initial stages of mycelium growth in plant tissues, but further development of the fungus in the host plant tissues is accompanied by the excess of phytohormones. Previously we have shown that *P. melonis* strain 502 synthesizes the ethylene phytohormone *in vitro*, and the cultural liquid of *P. melonis* strain 502 is characterized by growth-regulating properties and may inhibit or stimulate the growth of plants depending on the dilution and the test plant (Tsekhmister et al, 2021). It is generally known that many plant pathogens pretend to be mutualistic, and at first, they stimulate the growth of a host plant; artificially infected plants look

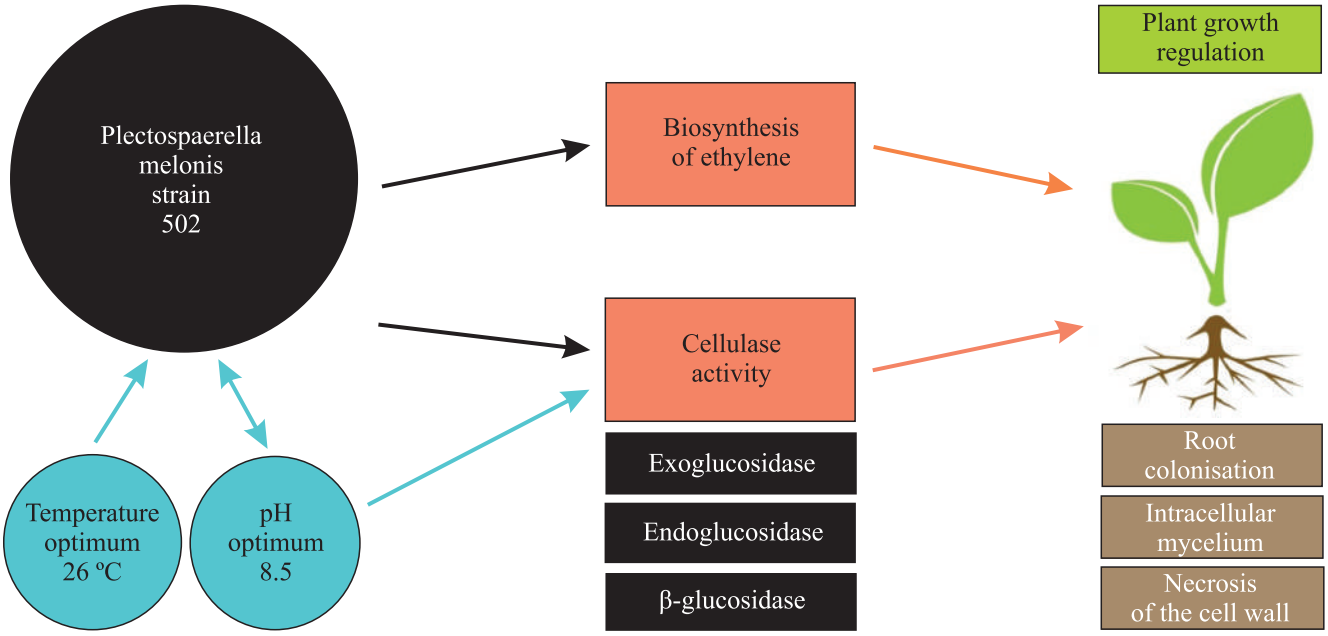

Fig. 3. Ecology of *P. melonis* 502

greener and healthy, then there are manifestations of the damage and even collapse of plants (van Baalen and Jansen, 2001; Bronstein, 2001). Numerous studies note that the microorganisms, endophytic for some plants, may be pathogens for others (Selim et al, 2012; Saikkonen et al, 2004; Kogel et al, 2006).

It has previously been noted that *P. melonis* is pathogenic only for Cucurbitaceae plants; it was very frequently isolated from the plants of other families as an endophyte and capable of limited colonization of the roots of some dicotyledonous plants of Asteraceae, Fabaceae, Malvaceae, Poaceae, and Solanaceae (Bruton et al, 2000b; Garsia-Jimenez et al, 1994; Armengol et al, 1998). However, recent studies have demonstrated that *P. melonis* is a pathogen for pepper, tomatoes, basil, and parsley (Carlucci et al, 2012; Raimondo and Carlucci, 2018 a, b).

Studying the sensitivity of Cucurbitaceae plants to the Spanish isolates of *P. melonis*, Armengol found that cucumber plants were in the range from resistant to highly susceptible (Armengol et al, 1998). In his turn, Bruton, studying the pathogenicity of American isolates of *P. melonis*, referred cucumber families to the highly resistant group; among the representatives of Cucurbitaceae there were no plants with a high level of susceptibility regarding the American isolates (Bruton et al, 2000b), which is in agreement with our data.

The resistant varieties in our study grew additional roots above the diseased place which is in agreement with the results of Iglesias et al (1999, 2000), who showed that a resistant variety of muskmelon Pat 81 was characterized by rather a developed and branched root system that promoted the formation of new roots, and found a higher number of additional roots, coming from hypocotyl above the diseased areas. Biernacki et al (2001) noted that *P. melonis* induced the worst damage to small roots, and the area of the root system was decreased, not affecting its mass.

Previously we have shown that *P. melonis* 502 is capable of active synthesis of cellulase enzymes (exo-, endocellulases and β-glucosidases), which trigger the degradation of the cellular wall of the plant (Patyka et al, 2022). It allows the fungus to penetrate inside cells and create the intracellular mycelium. Mainly, the epiderma and parenchymatous tissues of the root are damaged, and their ruination occurs at the effect of cellulase enzymes (Patyka et al, 2022). We have also shown the growth-regulating activity of *P. melonis* 502 and its ability to synthesize a phytohormone, ethylene (Tsekhmister et al, 2021). Secondary metabolites, studied by us, may be pathogenicity and virulence factors, conditioning the development of the disease. The study of *P. melonis* pathogenesis is still an urgent issue, though the first steps toward the investigation of its virulence and pathogenicity factors have already been made (Fig. 3). Generally, the incubation period of the disease and the susceptibility of plants may vary depending on the environmental conditions, especially temperature and pH, which affect not only the growth and development of a microorganism, but also its synthesis of biologically active substances (Kopilov et al, 2021; Patyka et al, 2022).

CONCLUSIONS

The phylogenetic analysis demonstrated that *P. melonis* strain 502 had 99 % homology to 10 isolates of *P. melonis* from the USA, Japan, and Italy. The percentage of homology with Spanish isolates, first isolated as the representatives of this genus, was lower (98 %). The root collar was damaged the most, which also indicated the similarity of symptoms to the American isolates compared to the ones from Spain, where the hypocotyl was damaged. There were no above-ground disease symptoms. The distribution of the disease and the degree of the damage were different depending on the variety, and did not depend on the plant maturity group, which demonstrated individual varietal sensitivity and the relevance of selecting resistant varieties as one of the main ways to control this pathogen. The study of the varietal sensitivity of *C. sativus* to *P. melonis* strain 502 showed that varieties Konkurent and Rodnichok could be referred to the highly resistant group, while Zhuravlionok, Liosha, and Nizhynskyi 12 – to moderately resistant ones. The distribution of the disease for varieties Zhuravlionok, Konkurent, and Rodnichok was 50 %, for varieties Nizhynsky 12 and Liosha – 60 and 80 %, respectively. Additional roots above the damaged area were observed in cucumber plants of varieties Zhuravlionok, Konkurent, and Rodnichok as a defensive response to being infected by the pathogen. No response of oversensitivity was registered in the investigated varieties of cucumber plants regarding *P. melonis* strain 502, which most probably was conditioned by the stability of the environmental factors and absence of abiotic stressors for the plant, including moisture shortage and high temperatures.

Adherence to ethical principles. All the experiments, described in this paper, did not involve animals.

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Філогенія *Plectosphaerella melonis* strain 502 і сортова чутливість рослин огірків

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Мета. Дослідити філогенетичні зв'язки *P. melonis* strain 502 та вивчити сортову чутливість рослин огірків до *P. melonis* strain 502. **Методи**. Екстракція ДНК проводили за допомогою полімеразної ланцюгової реакції (ПЛР). ПЛР проводили впродовж 35 циклів. Визначення отриманих ПЛР-продуктів здійснювали за допомогою сиквенування на автоматичному капілярному сиквенаторі Applied Biosystems ABI Prism 3130. Сиквенс гена 5.8S rRNA *P. melonis* strain 502 порівнювали з сиквенсами з GenBank database за допомогою BLAST analysis. Філогенетичний аналіз проводили методом Neighbor-Joining. Еволюційні відстані, розраховані за допомогою методу Jukes & Cantor. Еволюційний аналіз проведили у MEGA7. Чутливість рослин огірків визначали у вегетаційному досліді на штучному інфекційному фоні (ШІФ), який створювали шляхом внесення у ґрунт інфекційного матеріалу гриба *P. melonis* strain 502. Інфекційний матеріал вносили з розрахунку 50 тис. КУО/1 г грунту. Ураження кореневої системи оцінювали через 14 діб вирощування рослин на штучному інфекційному фоні. Індекс тяжкості захворювання (DSI) розрахований для визначення загальної чутливості досліджуваних сортів. Сорти, які отримали DSI < 2.0 вважалися високостійкими, від 2.0 до 2.9 помірно стійкими, сприйнятливі від 3.0 до 3.9 та 4.0 або вище дуже сприйнятливі. Для оцінки достовірності експериментальних даних використовували статистичні методи та Statistica 12 (Stat-Soft Inc., USA). Для оцінки індексу тяжкості захворювання розраховували середнє арифметичне та стандартне квадратичне відхилення (SD) при рівні значення $p < 0.05$. **Результати**. Використовуючи ПЦР метод, було отримано сиквенс *P. melonis* strain 502 довжиною 317 bp та проведено філогенетичний аналіз. Пошуки BLASTn у GenBank показали, що сиквенс ITS *P. melonis* strain 502 мав 99 % гомології з 10 ізолятами *P. melonis*. Каліфорнійський ізолят CA-1103 відрізнявся від *P. melonis* strain 502 лише 1 парою нуклеотидів. Відсоток гомології також становив 99 % з техаськими (TX-1060 і TX-1065), японськими (CBS 489.96 і ACCC:39138) та італійськими (Plect 211 і Plect 212) ізолятами. Іспанські ізоляти (A-943, A-537, A-99), мали менший відсоток гомології – 98 %. Поширення захворювання для сортів Журавльонок, Конкурент і Роднічок було 50 %, для сортів Ніжинський 12 і Льоша – 60 і 80 % відповідно. Дослідження сортової чутливості (рис. 2, табл. 2) до *P. melonis* strain 502 показало, що сорти Конкурент (DSI – 1.3 ± 0.0) і Роднічок (DSI – 1.5 ± 0.1) віднесено до групи високостійких, Журавльонок (DSI – 2.6 ± 0.3), Льоша (DSI – 2.2 ± 0.2) та Ніжинський 12 (DSI – 2.5 ± 0.2) – помірностійких. Найбільш уражена була коренева шийка, яка мала коричневий колір. Наземні симптоми були відсутні. У рослин огірків сортів Журавльонок, Конкурент і Роднічок спостерігали появу додаткових коренів над місцем ураження. **Висновки.** Філогенетичний аналіз показав, що *P. melonis* strain 502 мав 99 % гомології з

10 ізолятами *P. melonis* з США, Японії та Італії. З іспанськими ізолятами, які були вперше виділені як представники цього роду, відсоток гомології був нижчим (98 %). Найбільш уражувалась коренева шийка, що також вказує на подібність симптомів з американськими ізолятами, аніж іспанськими, у яких уражувався гіпокотиль. Наземні симптоми захворювання були відсутні. Поширення захворювання та ступінь ураження різнились залежно від сорту, і не залежило від групи стиглості, що свідчить про індивідуальну сортову чутливість і вказує на важливість селекції стійких сортів, як один з основних способів контролю цього збудника. Досліджені сорти огірків віднесено до груп високостійкі та помірностійкі. Поширення захворювання також різнилось залежно від сорту (Журавльонок, Конкурент і Роднічок – 50 %, Ніжинський 12 і Льоша – 60 і 80 % відповідно). У рослин огірків сортів Журавльонок, Конкурент і Роднічок спостерігали появу додаткових коренів над місцем ураження, як захисну реакцію на ураження патогеном. Не було зафіксовано реакції надчутливості досліджуваних сортів рослин огірків щодо *P. melonis* strain 502, що обумовлюється сталістю умов зовнішнього середовища та відсутністю абіотичних стресорів для рослини, таких як нестача вологи та високі температури.

Ключові слова: *Plectosphaerella melonis*, *Cucumis sativus*, ґрунтова хвороба, патоген, філогенетичний аналіз.

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