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**INFLUENCE OF ENDOPHYTIC AND SAPROTROPHIC
PENICILLIUM FUNICULOSUM STRAINS ON RESISTANCE OF
GLYCINE MAX L. UNDER SALT STRESS**

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Abstract. *Endophytic fungi almost didn't affect on the growth and characterized by wide biodiversity and accumulation biomass of soybean roots formed an important component of plant under both investigated NaCl ecosystems. Endophytic fungi play a concentrations. Thus, endophytic and significant role in the growth and saprotrophic P. funiculosum strains belong to the same species of fungi, development of plants under biotic and potentially produce the same spectrum of abiotic stresses. Influence of endophytic and saprotrophic Penicillium biologically active metabolites, but each funiculosum strains on resistance of in its econiche plays a certain ecological Glycine max L. under salt stress was role (mutualism or neutral), and studied. It was shown that under salinity therefore have different mechanisms of stress (70 mM and 140 mM NaCl, co-existence and interactions with plant and respectively) endophyte P. funiculosum soil microbiota. significantly promoted soybean growth attributes (shoot and root length, root fresh/dry biomass) in comparison to control plants. In contrast to the endophyte, saprotroph P. funiculosum*

Keywords: *endophyte, saprotroph, Penicillium funiculosum, soybean, salt stress*

Endophytic fungi are the widespread group of fungal symbionts associated with tissues of host plants without causing any symptoms of disease [2, 7, 12, 18]. Endophytic fungi

characterized by wide biodiversity and formed an important component of plant ecosystems. The endophyte-host association depends on plant-microbial interactions, may be parasitic, mutualistic

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or neutral and may switch with changes of host plant and environmental conditions [5, 18]. Endophytic fungi play a significant role in the growth and development of plants through different mechanisms. Endophytic fungi (being inside the host plant) produce a wide array of bioactive metabolites which can protect host plant under biotic and abiotic stresses [6, 7, 9, 12, 19]. Some endophytes have the ability to synthesis gibberellins, auxins (IAA) and abscisic acid (ABA) and this potential can be an arsenal for enhance resistance under unfavorable environmental conditions [6–8, 12, 21].

Salinity, especially sodium chloride, is main abiotic stress that negatively affects the development of plants (photosynthesis and diffusion of CO₂) and their productivity. It is estimated that about 50% of the arable land will be affected by salinity stress by the year 2050 [15]. According to estimates of the Food and Agriculture Organization of the United Nations (FAO) in Ukraine in 2017 the area of soil salinity is 2.8 million hectares and continues to grow [4, 16]. Endophytic fungi synthesize biological active metabolites, including phytohormones, and promote plant resistance to abiotic stress. Known that *Penicillium funiculosum* Thom is isolated as endophyte of many plants, in particular soybean (*Glycine max* L.) [8, 13]. Soybean is an important agricultural plant with high content of protein 38-

45%, oil 17-25%, lecithin - 1-2%, vitamins 5-6%; and therefore it's the world's leading source of protein and oil. Our previous studies have shown that *P. funiculosum* strains produced gibberellins, IAA, cytokinins and ABA [22, 24]; these data are in agreement with results of other scientists [8]. Khan et al. have been shown that endophyte *P. funiculosum* LHL06 synthesized physiologically active gibberellins, IAA and ABA, and promoted the growth of soybean under salt stress [8].

The aim of this work was to study an influence of endophytic and saprotrophic *P. funiculosum* strains on soybean under salt stress.

Materials and methods. The objects of the research were endophytic strain *P. funiculosum* 16795, isolated from cranberry leaf (Zhytomyr region) and saprotrophic strain 16790, isolated from chernozem soil (Dnipropetrovsk region). Strains are maintained in the collection of cultures of microscopic fungi of the Department of physiology and taxonomy of micromycetes of D.K. Zabolotny Institute of Microbiology and Virology National Academy of Sciences of Ukraine.

Strains were identified by a combination of culture and morphological characteristics and by DNA barcoding method. For sequencing, ITS region of the rDNA was amplified by Sanger using primers ITS1 and ITS4 [23]. The result of the BLAST search program revealed that the strains were

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identified as *Talaromyces funiculosus* (anamorph *Penicillium funiculosum*) and were submitted to the GenBank with accession numbers KY620212 and KY865175, respectively.

The influence of *P. funiculosus* strains from different ecoiniche on soybean (*Glycine max* L.) under salt stress was studied by Khan et al. method with some modifications [8, 11]. Soybean seeds were surface sterilized using McCutcheon and Carroll method to exclude the effects of other microorganisms [14]. After that, the seeds were germinated for 2-3 days in Petri dishes on filter paper moistened with autoclaved distilled water at temperature $26 \pm 2^\circ\text{C}$. Germinated seeds (length of seedlings 0.7-1.0 cm) were treated with inoculum of *P. funiculosus* strains for 1 hour. The inoculum was grown under submerged conditions as described previously [22]. The treated seeds were placed in pots with sterile sand and grown chamber (day/night cycle: 16 h/ 26°C ; 8 h/ 18°C) for 14 days. Sand (particle size 1.0 mm, pH 7.0) washed three times with distilled water, dried and put in pots (200 g per pot). Then sand was sterilized twice at 120°C for 15 min. Soybean plants were treated under salt stress (70 mM and 140 mM NaCl solutions) for 7 days, and each pot received 1400 ml of salt solution.

The experimental design had nine sets of soybean plants: the control (without inoculation with fungi or NaCl solutions); plants treated with fungal

inoculum of endophytic and saprotrophic *P. funiculosus* strains; control and fungal inoculums treated plants, treated with 70 and 140 mM NaCl solutions. The growth parameters i.e. shoot and root length, fresh and dry biomass were studied as percentage of control. The seed, treated with sterilized distilled water was used as control. All variants of the experiment were performed in triplicate. Statistical analyses of data were carried out using the Microsoft Excel software.

Results and discussion. Shoot and root length of endophyte inoculum treated soybean increased up to 24 and 28% in comparison to control and saprotroph inoculum treated - 4-5%, respectively (Fig. 1). Shoot and root length of control plants under 70 mM NaCl solution decreased by 24 and 35% in comparison to control and under 140 mM - by 32-42%, respectively. Inoculation with endophyte affected positive on treated plants under salt stress. In particular, the shoot and root length of plants under 70 i 140 mM NaCl solution increased to 17-28%. However, the effect of the saprotroph wasn't significant - the shoot and root length of soybeans didn't exceed 4-8% in comparison to control. Consequently, the endophytic *P. funiculosus* strain stimulated the shoot and root growth of soybean in both cases (control plants inoculated with endophyte and the ones under salt stress).

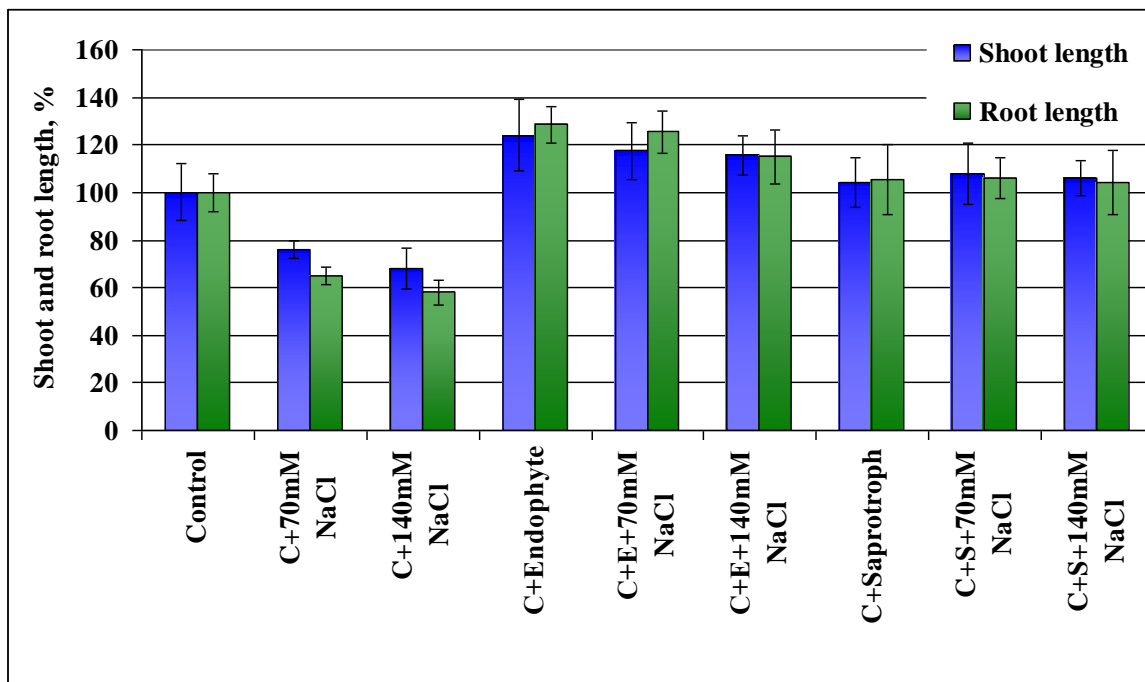


Fig. 1. Effects of salt stress (70 i 140 mM) on the shoot and root length of control soybeans and inoculated with endophytic and saprotrophic *P. funiculosum* strains: E – endophyte, S – saprotroph, C – control plant.

It is known that the root is the most sensitive to stress part of plants, so we pay special attention to this parameter (Fig. 2). Inoculation plants with endophytic *P. funiculosum* strain caused significant changes in biomass accumulation – root fresh/dry weight (40 and 27% respectively), however plants treated with saprotrophic strain accumulated less biomass (5 and 7%). It was shown that root fresh/dry root weight of control plants under 70 mM NaCl solution decreased at 14-36% in comparison to control and in case of 140

mM salt stress – 23-44% respectively. Increased root fresh/dry weight were detected at 23 and 26.4% for endophyte inoculated plants treated with 70 mM NaCl. Also endophyte *P. funiculosum* strain stimulated accumulation biomass of soybean roots by 15-16.4% under 140 mM NaCl solution. In contrast to the endophyte, saprotroph *P. funiculosum* almost did not affect on the growth and accumulation biomass of soybean roots (4-7%) under both investigated NaCl concentrations.

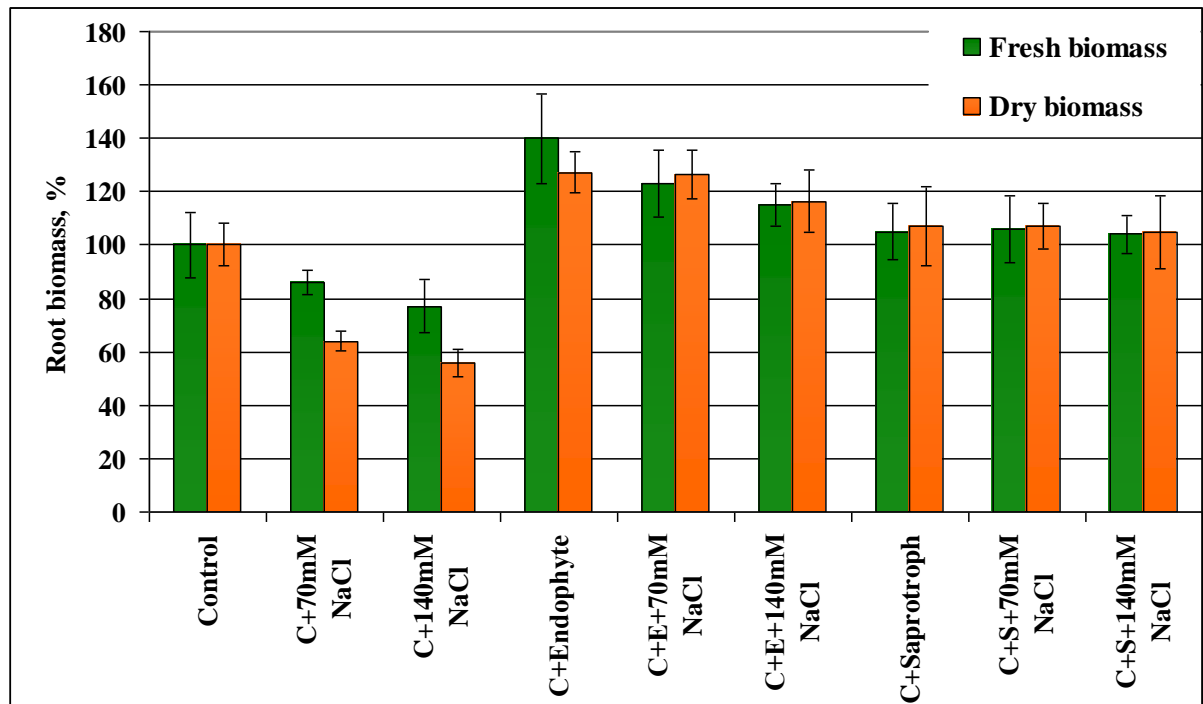


Fig. 2. Effects of salt stress (70 i 140 mM) on the root fresh/dry weight of control soybeans and inoculated with endophytic and saprotrophic *P. funiculosum* strains: E – endophyte, S – saprotroph, C – control plant.

Based on the obtained data, we can conclude that the endophyte *P. funiculosum* strain stimulated roots biomass accumulation of soybean plants and reduced the negative influence of salt stress.

Nowadays more attention is paid to the study of endophytic fungi and synthesis by them a large number of biologically active substances that protect and help to survive the host plant under adverse environmental conditions. [8, 10, 12, 18, 20]. It is known that endophytic strains of genus *Penicillium* form a spectrum of phytohormones (gibberellins, IAA, cytokinins and ABA), which gives them an advantage over other types of micromycetes [8, 10, 12, 20].

A strain of *P. citrinum* isolated from *Ixeris repenes* (L.) A. Gray was described for the first time as a possible advantage for the plants at saline environment [7]. *P. citrinum* promote *I. repenes* growth by production of bioactive gibberellins. It is known that endophytic strains of genus *Penicillium*, such as *P. citrinum*, *Penicillium* sp. MH7, *Penicillium* sp. LWL3, *P. minioluteum* LHL09, *P. janthinellum* LK5 and *P. funiculosum* synthesize physiologically active and inactive forms of gibberellins, which according to the authors promote the growth plants to re-programmed soybean to high growths under salinity stress by secretion of endogenous phytohormones [6–9, 20]. Also, *P. minioluteum* LHL09, isolated

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from *Glycine max* L. synthesize of bioactive gibberellins GA₄ and GA₇ under salt stress and stimulate plant growth [1]. Endophyte *P. resedanum* LK6, isolated from *Capsicum annuum* L., was also reported significantly promoted pepper plants growth attributes (shoot length, shoot fresh/dry biomass, chlorophyll content and photosynthesis rate) in comparison to control treatments [10]. In addition, strain *P. resedanum* LK6 synthesized gibberellins GA₁ and GA₄, which according to the authors, reduced the negative effect of abiotic stress.

It has been shown that the endophyte *P. funiculosum* enhanced soybean seed germination and reduced the negative effects of salt stress by stimulating soybean growth [8]. Endophyte *P. funiculosum* produced gibberellins (GA₁ 1.53 ng/ml; GA₄ 9.34 ng/ml; GA₈ 1.21 ng/ml; GA₉ 37.87 ng/ml); indole acetic acid 14.85 µg/ml and low level of ABA under salinity induced abiotic stress (70 and 140 mM). Thus, the amount of ABA in plants incubated by the endophyte was 671.32 ng/g, however, sterile plants were characterized by a higher concentration of this hormone 1689.05 ng/g. ABA involved in regulation of the signaling pathways in the growth and development of plants under abiotic stress. In plant ABA level increase because promotes stomatal closure to minimize water loss and then mediates stress damage through activation of many stress-responsive

genes, which collectively increases the plant's stress tolerance [9].

It was established that investigated *P. funiculosum* strains synthesized bioactive gibberellins (GA₃, GA₄ and GA₇), auxins, cytokinins and ABA, which is consistent with the literature data [6–8, 10, 12, 20, 22, 24]. Endophyte and saprotroph strains have been identified as one species - *P. funiculosum* by morphological, physiology and molecular genetic features [22]. Investigated strains produce the same complex of phytohormones, but micromycetes, isolated from different ecosystems, differently influenced on growth and development of soybean plants under salt stress. Thus, the endophyte *P. funiculosum* promoted growth of soybean shoot and root, root fresh/dry biomass. In contrast, saprotrophic fungus practically did not affect the growth and development of the plant under abiotic stress.

We assume that the strategies and mechanisms of action of the endophytic and saprotrophic *P. funiculosum* strains on plants under abiotic stress differ. The endophyte is inside the host plant in more favorable living conditions in comparison with saprotroph both in terms of availability of sources of nutrition and competition with other microorganisms; at the same time, it forms a mutualistic relationship with the plant. Saprotroph can't to compete colonize the rhizosphere of plants and it acts more as an associate of plants which

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fed with root extracts, products of hydrolysis of plant residues, although it synthesizes a number of substances with antibiotic activity against some soil microorganisms [3, 17]. Thus, endophytic and saprotrophic *P. funiculosum* strains belong to the same species of fungi, potentially produce the same spectrum of biologically active metabolites, but each in its niche plays a certain ecological role (mutualism or neutral), and therefore have different mechanisms of adaptation

to stress conditions, co-existence and interactions with plant and soil microbiota.

Conclusions. Thus, endophyte *P. funiculosum* have the ability to overcome the adverse effects of *Glycine max* L. under salt stress by the synthesis of the spectrum of plant growth regulators has been established and have further application in the development of modern "green" technologies for increasing the general resistance of plants to stress factors.

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ВПЛИВ ЕНДОФІТНОГО І САПРОТРОФНОГО ШТАМІВ *PENICILLIUM FUNICULOSUM* НА СТІЙКІСТЬ *GLYCINE MAX* L. ДО СОЛЬОВОГО СТРЕСУ

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Анотація. Ендофітні гриби характеризуються широким біорізноманіттям та утворюють важливу складову екосистем, що пов'язана з рослинами. Ендофіти відіграють важливу роль у рості і розвитку рослин, що забезпечують захист рослин від біотичних та абіотичних стресів. Вивчено вплив ендофітного та сапротрофного штамів *Penicillium funiculosum* на стійкість *Glycine max* L. до сольового стресу. Показано, що за дії сольового

стресу (70 мМ та 140 мМ NaCl відповідно) ендофіт *P. funiculosum* значно підвищував ростові параметри сої (довжина паростків і коренів, волога та абсолютно суха біомаса коренів) порівняно з контрольними рослинами. На відміну від ендофіта, штам-сапротроф *P. funiculosum* майже не впливав на ріст і накопичення біомаси коренів сої за дії обох досліджених концентрацій NaCl. Таким чином, ендофітний і сапротрофний штами *P. funiculosum* належать до одного виду, потенційно можуть продукувати однаковий спектр біологічно активних метаболітів, проте кожний у своїй еконіші відіграє певну екологічну роль (мутуалізм або нейтральна), а тому мають різні механізми пристосування до умов існування за дії стресів, співіснування та взаємовідносин з рослиною і мікробіотою.

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Ключові слова: *ендофіт, сапротроф, Penicillium funiculosum, соя, соловий стрес*

ВЛИЯНИЕ ЭНДОФИТНОГО И САПРОТРОФНОГО ШТАММОВ *PENICILLIUM FUNICULOSUM* НА УСТОЙЧИВОСТЬ *GLYCINE MAX* L. К СОЛЕВОМУ СТРЕССУ

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Аннотация. Эндоефитные грибы характеризуются широким биоразнообразием и образуют важную составляющую экосистем, связанную с растениями. Эндоефиты играют важную роль в процессах роста и развития растений, способствуют защите растений от биотических и абиотических стрессов. Изучено влияние эндоефитного и сапротрофного штаммов *Penicillium funiculosum* на устойчивость *Glycine max* L. к солевому стрессу. Показано, что под действием солевого стресса (70 мМ и 140 мМ NaCl соответственно) эндоефит *P. funiculosum* значительно повышал ростовые параметры сои (длина проростков и корней, влажная и абсолютно сухая биомасса корней) по сравнению с контрольными растениями. В отличие от эндоефитного, штамм-сапротроф *P. funiculosum* почти не влиял на рост и накопление биомассы корней сои под действием исследованных концентраций NaCl. Таким образом, эндоефитный и сапротрофный штаммы *P. funiculosum* принадлежат

к одному виду, могут синтезировать одинаковый спектр биологически активных метаболитов, однако каждый в своей эконше играет определенную экологическую роль (мутуализм или нейтральная), а потому имеют разные механизмы приспособления к условиям существования под действием стрессов, сосуществования и взаимоотношений с растением и микробиотой.

Ключевые слова: *ендофит, сапротроф, Penicillium funiculosum, соя, солевой стресс*