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FORMATION AND FUNCTIONING OF CHAETOMIUM COCHLIODES / FAGOPYRUM ESCULENTUM ENDOPHYTIC ASSOCIATION

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ABSTRACT

Many representatives of soil saprotroph fungi can penetrate into plant tissues forming endophytic associations with a positive effect on both micro- and macroorganisms. The endophytic fungi may positive act on growth processes, immune status, and resistance of plants to stress factors. For some crops, including buckwheat, the ability to form endophytic associations with soil fungi remains unexplored. Thus the main objectives of our study were to establish the features of interaction between *Chaetomium cochliodes*3250 and buckwheat plants. Conventional biochemical (content of plant growth regulators, activity of succinate dehydrogenase, acid and alkaline phosphatase, exoglucanase, endoglucanase, β-glucosidase, polygalacturonase) and physicochemical (content of photosynthetic pigments) study methods were used during the work. We established the capability of *C. cochliodes* 3250 for growth-regulating substances synthesis. The fungus could produce indolyl 3-acetic acid, gibberellic acid, that stimulates growth and development of plants both with mediator molecules, synthesizes 2,4-epibrassinolide, cholesterol and ergosterol playing an important role in plant resistance against pathogens. We found out that *C. cochliodes* 3250 was capable for active synthesis of cellulase complex enzymes (exoglucanase, endoglucanase, β-glucosidase) and polygalacturonase, necessary for fungus penetration into the root tissues. Pre-sowing treatment of buckwheat seeds by fungus caused the main physiological responses of plants: an increase of total adsorption and active working surface of the roots, the length and weight of plants, the leaf area and the content of chlorophylls *a* and *b*. Thus, the ability of *C. cochliodes*3250 to form an efficient endophytic association with buckwheat plants been improved.

Keywords: soil fungus *Chaetomium cochliodes*, buckwheat, endophytic association

INTRODUCTION

In recent decades, along with the mycorrhizal symbiosis, significant attention of researchers was paid at another scantily studied process, namely endophytism of soil saprotrophic fungi to the plant roots. The metabolic and growth processes activated more than usual ones, the immune status is enhanced and stress resistance is raised up in plants infected with endophytic fungi (Arnold, Harre,2003; Waller et al., 2005; Marquez et al., 2007; Antonyak et al., 2013). In turn, the localization of endophytes in plant tissues protects them from adverse environmental conditions, while they do not compete with rhizosphere microflora and have direct access to nutrients synthesized by plants. The formation of endophytic systems is not a prerequisite for plant development. In addition, fungi can exist for a long time in plant tissues without any signs of their presence and become to evince their activity under adverse environmental conditions: moisture and mineral nutrient deficiencies, the negative effects of environmental factors (Lutova et al., 2000; Spatafora et al., 2007). *Acremoniun, Alternaria, Aspergillus, Chaetomium, Cladosporium, Claviceps, Collectotrichum, Cryptococcus, Curvularia, Diaporthe, Epicoccum, Fusarium, Geomyces, Glomus, Leptospora, Microdochium, Neotyphodium, Paecilomyces, Penicillium, Phaeomoniella, Piriformospora, Rhizoctonia, Rhizopus, Rhodotorula, Sarocladium, Talaromyces, Trichoderma, Wallemia* and *Xylaria* are mentioned as endophytes of the crops majority (Charest et al., 1993; Cevnik et al., 2000; Tsavkelova et al., 2005; Subramanian, Charest, 1997; Hause, Fester, 2005; Ding et al., 2005; El-Zayat, 2008; Hata et al., 2010; Kopylov, 2013; Singh, 2017; Rana,Kour et al., 2016; Rana, Yadav et al., 2016, Yadav et al., 2017; Spagnoletti et al., 2016). The search for symbiotically efficient endophytic fungi and establishment of their features according to interaction between plants and soil fungi are still in the focus of intensive studies. Despite the significant amount of fungi capable to form mycorrhizal or endophytic symbiosis, many researchers investigated a large number of plant abilities to form associative systems with micromycetes that had not been established. One of such important crops beingis buckwheat plants.J.L.Harley and E.L.Harley (Harley, Harley, 1987), J.P.Gai et al. (Gai et al., 2006), B.Wang and Y.L.Qiu (Wang, Qiu, 2006) could not find any signs of buckwheat plantscolonization with mycorrhizal fungi. At the same time, the first results of molecular genetic analysis with fungi isolated from edaposphere and rhizosphere, the sequenced DNA fragments found to be close to *Glomus*species (Likar et al., 2008).Therefore the issue of mycorrhizal endophytic symbiosis formed with buckwheat is controversial and has not been completely established.
At the early stages of vegetation, buckwheat plants are particularly susceptible to nitrogen and phosphorus intakes; the lack of these elements in mineral nutrition of plants can cause delay in growth and the root system development. Providing buckwheat with all necessary elements is possible due to mineral fertilizers. Another way for the environment to stay safe is the usage of endophytic fungi in root zone forming plant-microbial associations.Thus it is important and timely to search for endophytic saprotrophic fungi that are able to survive in sowing buckwheat root zone and affect positively for the yield and products quality.
Chaetomium is one of the largest genera of the *Chaetomiaceae* family, which is represented by more than 100 species (Zhang and Soytong, 2013). An active biological control agents were identified among the fungi of this genus; they tread down the growth of bacteria and fungi by direct competition, micro-parasitism or antibiosis (Sibounnavongetal., 2011). A large number of the *Chaetomium* metabolism biologically active products are known. Micromycete *C. atrobrunneum* L. M. Ames produces fungicides fuscoatroside and heteatrosine (Hwangatal., 2000; Kobayashi et al., 2005), *C. cochliodes* Palliser – antibiotic hetamine (Geigeretal., 1944), *C. globosum* Kunse - phytotoxinazophilone and hematomohiline D and J, antibiotics Hetamine and Hetirovidine (Piyasena et al., 2015). *C. gracile* Udagawa is an antimicrobial hetochromin A (Bai et al., 2015).
It is known, that soil saprotrophic fungus *Chaetomium cochliodes* 3250 introduced into the root zone of spring wheat and soybeans, develops actively on the plants roots and forms fruiting bodies on the surface of roots and root hairs. Moreover, it penetrates into the rhizoderm cells, indicating the formation of endophytic associations. (Kopilov, Nadkernichny, 2008; Kopylov et al., 2010).

Therefore, the focus of our studying was the interactions between macro- and microsymbionts in endophytic association of *Chaetomium cochliodes* 3250 /*Fagopyrum esculentum.*

MATERIALS AND METHODS

The fungus strain

Chaetomium cochliodes Palliser 3250 is a fungus strain with antagonistic activity taken from useful soil micro-organisms collection at The Institute of Agricultural Microbiology and Agro-Industrial Production NAAS (Volkogon *et al*., 2015).
The *C. cochliodes* 3250 fruiting bodies are located on the mycelium surface and grow in large numbers. They are round, oval, with their colour ranging from dark green to dark brown, with plant tillers and rhizoids at the bottom. Bags are club-shaped and octosporous. Ascospores are oval and lemon-shaped.

The fungus cultivation for growth regulating substances determination

C. cochliodes 3250 was cultivated within 18 days at a temperature of +25-26°C in liquid medium (Belyavskaya et al., 2009). The pH value was 7,0. After the fermentation the fungus titer came to (3-4) × 10^5 colony forming units (CFU) in 1 ml of the cell culture fluid.

Growth regulators content determination

The liquid medium was evaporated to dryness in the vacuum. Then we made triple extraction using methanol/acetonitrile mixture (1:1). The obtained extractions were mixed up and evaporated in the vacuumat at the temperature of +45 °C.
Auxins and cytokines determination were carried out using method of spectrodensitometric thin-layer chromatography. Phytohormones detection was made via scanning spectrosensometer "Sorbphil" (Russia). The number of synthesized phytohormones was calculated in μg/ml of fungus liquid medium.
The quantitative content of gibberellins and brosinosteroids was made via high-pressure efficient chromatography method (liquid chromatography Aligent Technologies 1200 (USA) with mass spectrometric detector Agilent Technologies G1956B (USA). The Zorbax SB-C18 chromatographic column (2,1 mm× 150 mm, 3 μm) (Aligent Technologies, USA) beenusedduringresearch (150 mm length, 4 mm diameter). The moving phase flowing through the column was 0,35 ml/min, the thermostat column temperature was 30°C, the volume of injections was 3 μl.Diode-matrix detector was used for registration at 198 and 210nm fixing absorption spectra at 190-400 nm. Fluorescent detector was used with 210 nm extinction waves and 410 nm with emissions. Moving phase – acetonitrile/water (75:25) Appropriate Sigma solutions (USA) were used as standards for comparison.

The fungus cultivation for cellulosic and polygalacturonase activity determination

C. cochliodes 3250 was cultivated within 12 days at a temperature of +25-26 °C in modified Czapek liquid medium (g/l: $(NH_4)_2HPO_4$ – 2,5; K_2HPO_4 – 1,0; $MgSO_4$ – 0,5; KCl – 0,5; $FeSO_4$ – 0,01). The strip of filter paper (50 mg) was used as the only source of carbon. pH value was 7,0. The spore suspension with a caption of 1×10^6CFU was used for seeding at the amount of 5% to the nutrient medium volume. The fungus conidia and hyphae fragments for *C. cochliodes* 3250 seed material was washed off from the wort-agar tubes. After the biomass cultivation it was separated with filtering through the porcelain sieve. The obtained filtrate was used for carrying out the tests.

Cellulosic complex components activity test

The accounting was made on 3, 6, 9 and 12 days of cultivation. The Czapek medium without fungus was used as a control variant. Exoglucanase, endogluconase and β-glucosidase were enzymes of the cellulase complex. The unit of exoglucanase activity was the quantity of enzyme capable for forming of 1 mg reducing sugars. The units of endogluconase β-glucosidase were the quantity of enzymes capable to form the 1 mg reducing sugars for 30 minutes. As a substrate we used avicel in quantity of 50 mg (Sigma, USA), 1 ml 0,5% Na-CMCsolution (Sigma, USA), 1 ml 0,025% whole biosis solution (Sigma, USA). Sodium citrate buffer concentration was 0,05 M. The number of reducing sugars was detected with Somogy-Nelson method (Bilay, 1982). The test mixture was being incubated at 40°C. The standard glucose solutions were used for calibration curve building.

Material plant growing

The ability of *C. cochliodes* 3250 for forming associative system with buckwheat plants of Antaria sort was tested during vegetative experiment within 40 days using 2 dm^3 plastic vessels.
The type of soil was sod-medium podzolic sulfur-sandstone; the humus contents – 1,02%; the nitrogen contents – 54,9 mg/kg; the moving forms of phosphorus contents – 110-120 mg P_2O_5; the exchangeable potassium contents – 120-130 mg K_2O per 1 kg of soil; $pH_{salt.}$ – 5,2; pH_{water} – 6,0; Ca – 5,8, Mg – 0,61 mg·eq per 100 g of soil.
In control variant the seeds were moistened with water (1% by weight). Pre-sowing treatment of control seeds by *C. cochliodes* 3250 was made in quantity of 4×10^5CFU per 1 seed.
The seeds were planted to a depths of 2,0 cm. Each vessel contained 15 plants with the future thinning to 10.

Plant material preparing

The samples of roots were taken out at 20, 30, 40 day. Then the roots were washed with drain and sterile water, chopped and homogenized in 0,1 M phosphate buffer in the ratio of 1:10.

Succinatedehydrogenase activity determination

Succinatedehydrogenase activity (SDH) in control variant roots and in oculated plants with *C. cochliodes* 3250 was conducted using potassium ferriyanide (Resyapkin, Slyshkov, 2009). Because of the SDH actions, the succinate recovered potassium ferricyanide ($K_3[Fe(CN)_6]$), the solution of which had at first a yellow color, to a colorless potassium ferrocyanide ($K_4[Fe(CN)_6]$), with enzyme activity proportional to the amount of reduced ferricyanide.
A mixture of 20% trichloroacetic acid and 0.1 M phosphate buffer in a 1: 1 ratio was used as the optical control.
To determine potassium ferriyanide in tested samples we made a calibration curve (from 100 to 1000 μg of ferriyanide in 4 ml of sample solution).
The amount of ferricyanide that was recovered during incubation was calculated due to the difference in existences. As the reaction proceeds stoichiometrically, SDH activity was expressed by the amount of oxidized succinate and measured in nmolsuccinate / mg protein per 1 min. The 3-times biological and 3-times analytical replicates were used.

Acid and alkaline phosphatase activity

Acid and alkaline phosphatase activity in control variant roots and buckwheat plants inoculated with *C. cochliodes* 3250 were detected using photocolorimetry method, based on hydrolysis of glycerophosphate with phosphatase solution in the presence of the appropriate buffer (acid phosphatase – pH 5,2, alkaline phosphatase – pH 8,9)(Ermakov et al., 1972). The content of inorganic phosphorus in filtrate was detected by Lowry and Lopez method modified by Skulachev (Ermakov et al., 1972). The method is based on transformation of phosphoric molybdenum acid complex by ascorbic acid. Sensitization of the reaction was achieved by Cu^{2+} ions in incubative solution. According to the series of potassium dihydrogenphosphate solutions with different concentration calibration curve was built. Phosphatase activity was expressed in milligrams of inorganic phosphorus excretion by enzyme action on 1 g of tissue.

Physiological characteristics of buckwheat plants studying

General and working adsorption surface detection was made according to the Sabin and Kolosov method using methylene blue (Gorodniy et al., 2005).
Biometric indexes of plants were detected with measuring-weight method.
The area of photosynthetic surface was detected with the carving method (Tretyakov et al., 1990).
The maintenance of chlorophyll *a* and *b* were detected spectrophotometrically (Grodzinsky, 1973).

Statistical analysis

Calculations and statistical analysis of the obtained results were made according to generally accepted methods. Parametric criteria for normal distribution were used determining arithmetic mean and square deviation mean less than 0,05 significance level. Microsoft Excel were used for processing. To assess the reliability of the differences between variants the slightest significant difference (SSD_{05}) was calculated.

RESULTS

Growth-regulating substances of *C. cochliodes*3250

Quantification analysis of growth regulators in the cultural fluid of *C. cochliodes* 3250 showed that the fungus was capable to synthesize auxins and gibberellins (Table 1). It also was found that *C. cochliodes* 3250 produced 8.7 μg/mL of cultural fluid. Furthermore the fungus was capable to synthesize gibberellic acid 56.4 μg/mL of cultural fluid.
Cholesterol, ergosterol and 2,4-epibrassinolide were detected in the micromycete cultural fluid. The presence of cholesterol in the cultural fluid could be evidence

of its role as a precursor in the synthesis of 2,4-epibrassinolide (Mandava, 1998). It is known that ergosterol and 2,4-epibrassinolide contribute to increased resistance of plants to pathogenic microorganisms (Janitor, 2002). The high content of steroid derivatives indicates a high phytostimulating and protective activity of the fungus.

Table 1 Content of growth regulators in the cultural fluid of *C. cochliodes*3250

Phytohormonal compounds and their precursors	Content in CF, µg/mL
Indolyl-aceticacid	8.7 ± 0.003
Gibberellic acid	56.4 ± 2.12
2,4-epibrassinolide	0.01098 ± 0.0012
Cholesterol	7.06 ± 0.05
Ergosterol	17.88 ± 0.38

Cellulolytic complex of *C. cochliodes*3250

Different types of fungi – producers of cellulose – differ in the formation way of individual components of cellulolytic complexes. The data of hydrolytic activity of components of the cellulolytic system *C. cochliodes* 3250 are presented in Table 2.

Table 2 Changes in the activity of the *C. cochliodes* 3250 cellulolytic complex components

Day	Exo-Glucanase activity, units*	Endo-glucanase activity, units**	β-glucosidase activity, units**	Pectinase activity,units***
3	0,21 ± 0,003	0,2 ± 0,02	0,24 ± 0,03	2,21 ± 0,11
6	0,26 ± 0,02	0,21 ± 0,24	0,36 ± 0,02	2,81 ± 0,07
9	0,67 ±0,03	0,52 ± 0,02	0,54 ± 0,02	2,95 ± 0,02
12	0,42 ± 0,01	0,31 ± 0,02	1,02 ± 0,03	0,55 ± 0,02

* such amount of enzyme which forms 1 mg of reducing sugars in 60 min was taken as a unit of exoglucanase activity,
** such amount of enzymes which forms 1 mg of reducing sugars in 30 min was taken as a the unit of endoglucanase and β-glucosidase activity,
*** such amount of enzyme that catalyzes conversion of 1 g of pectin to galacturonic acid in 60 min was taken as the unit of pectinase activity.

The exoglucanase, endoglucanase and β-glucosidase activity was established in the cultural fluid of *C. cochliodes* 3250. The exoglucanase activity in the fungal cultural fluid was 0.67 U/mL at Day 9 of cultivation. The presence of exoglucosidase in the cultural fluid of the fungus indicated the ability of the *C. cochliodes* 3250 to degrade the crystalline form of cellulose. Endoglucanases provided hydrolysis of amorphous cellulose to cellobiose (endoglucanase activity of *C. cochliodes* 3250 was 0.52 U/mL at Day 9). β-glucosidase completed degradation of cellulose and provided hydrolysis of cellobiose to glucose (β-glucosidase activity of *C. cochliodes* 3250 was 1.02 U/mL at Day 12).
Pectinase activity of the cultural fluid of *C. cochliodes* 3250 determines the process of depolymerization of the adhesive layer of pectin between the adjacent walls of plant cells. The highest pectinase activity was recorded at Day of micromycetes cultivation and was 2.95 U/mL.

Activity of succinate dehydrogenase of endophytic association of *C. cochliodes* 3250 / *F. esculentum*

Changes in the activity of succinate dehydrogenase (SDH) of the roots of the buckwheat plants over time are shown in Figure 1. It has was determined that the activity of SDH at day 20 (branching phase) of inoculated plants exceeded the control parameters by 28 %, and at day 40 (flowering phase) – by 13 %. Further, there was no significant difference in the SDH activity of the control and experimental plants.

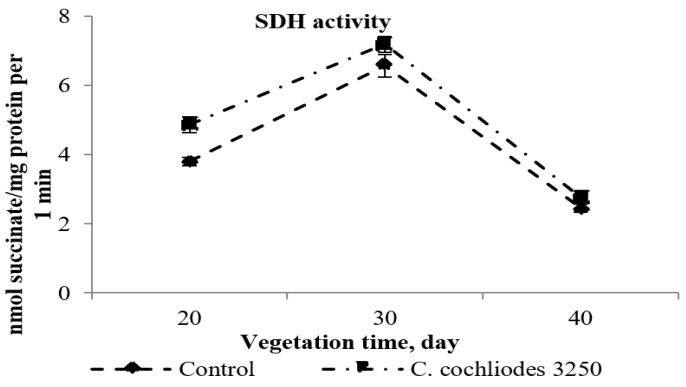

Figure 1 Activity of succinate dehydrogenase (SDH) in the roots of buckwheat under pre-sowing seed treatment by *C. cochliodes* 3250 (vegetative experiment)

Activity of acid and alkaline phosphatase of endophytic association of *C. cochliodes* 3250 / *F. esculentum*

The evaluation of alkaline phosphatase activity in mycorrhizal tissues is widely used as a parameter of the physiological efficiency of mycorrhizal infection. We found that under the treatment with *C. cochliodes* 3250, activity of alkaline phosphatase (ALP) in the buckwheat roots was higher in the comparison to control throughout the growing season. The data is presented in Figure 2.
In addition, in control plants, the activity of the enzyme decreased over time, and in the inoculated – increased. Similar changes over time were observed for acid phosphatase (AP). The data is presented in Figure 3.

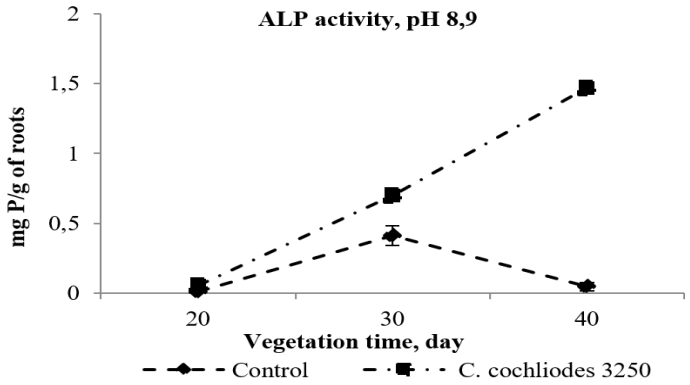

Figure 2 Activity of alkaline phosphatase (ALP) in buckwheat roots under pre-sowing treatment of seeds by *C. cochliodes*3250 (vegetative experiment)

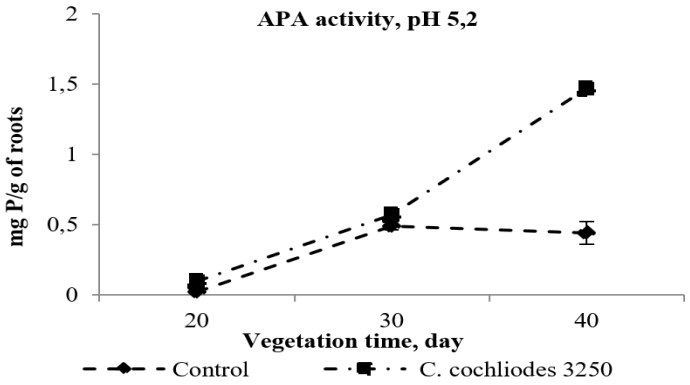

Figure 3 Activity of acid phosphatase (APA) in buckwheat roots under pre-sowing treatment of seeds by *C. cochliodes*3250 (vegetative experiment)

***C. cochliodes* 3250 influence on buckwheat plants**

The close interaction of plants with endophytic fungi significantly affects the structure of the root system. Pre-sowing treatment of buckwheat seeds by *C. cochliodes* 3250 contributed to an increase in the surface of the plants root system. In the branching phase (Day 20), the total adsorption surface of the roots of treated plants was 2.5 times higher than in the control ones, and in the flowering phase – 1.2 times (Figure 4). The area of the active working surface of the roots of the treated plants in the branching phase was at the control level, in the flowering phase – by 20 % higher (Figure 5).

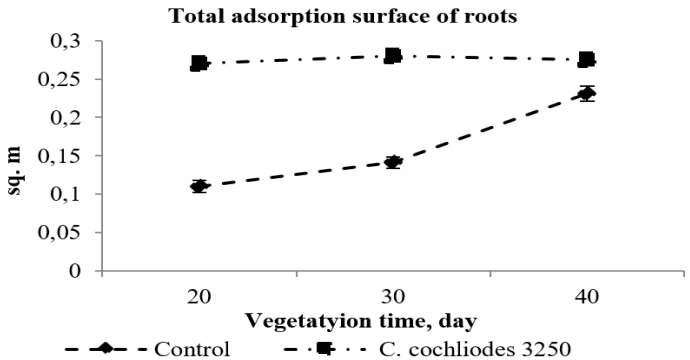

Figure 4 Total adsorption surface of root system in buckwheat plants under pre-sowing treatment of seeds by *C. cochliodes* 3250 (vegetative experiment)

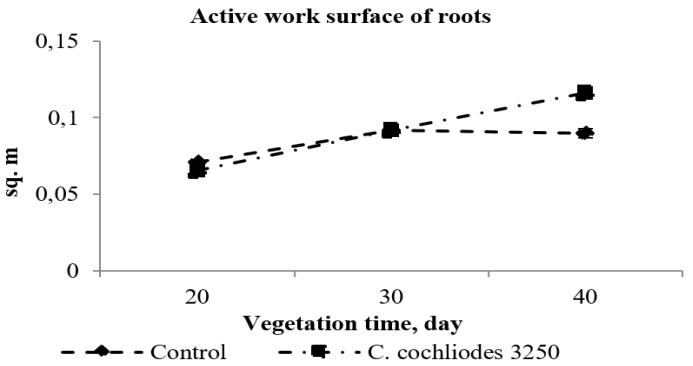

Figure 5 Area of active work surface of buckwheat plants root system under pre-sowing treatment of seeds by *C. cochliodes* 3250 (vegetative experiment)

The increase in the adsorption surface of the roots contributed to the supply of mineral substances and had a direct impact on the plant growth improvement and development during the vegetation period. For examples, under exposure to *C. cochliodes* 3250, the dry matter of experimental plants exceeded the control matter from 4 to 19 % at different stages of vegetation. The results are presented in Figure 6.

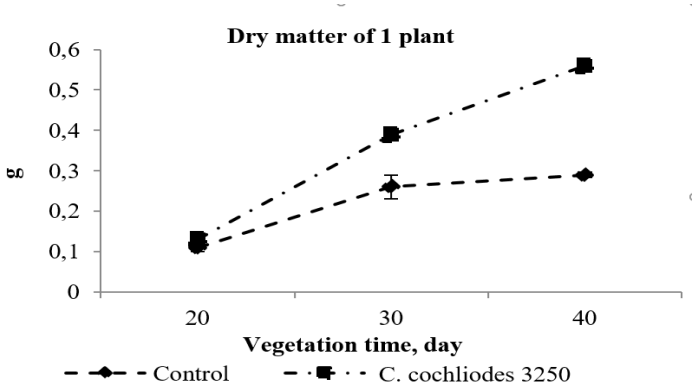

Figure 6 Dry matter of buckwheat plants under pre-sowing treatment of seeds with *C. cochliodes*3250 (vegetative experiment)

On the Day 20 of the experiment, the height of the experimental plants exceeded the control by 26 %; further, the difference was less noticeable, however the inoculated plants remained capable to grow actively (Figure 7).

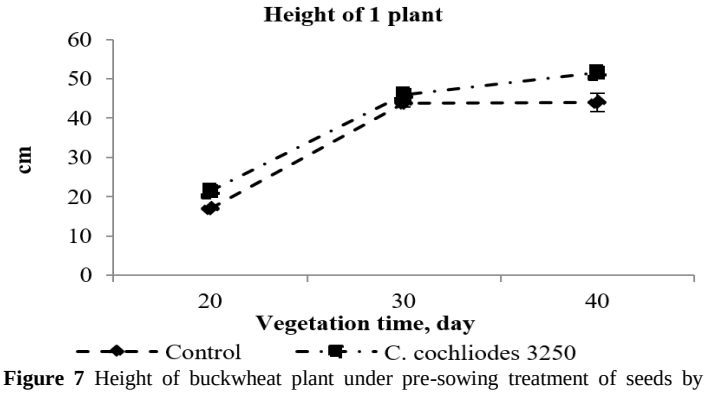

Figure 7 Height of buckwheat plant under pre-sowing treatment of seeds by *C. cochliodes*3250 (vegetative experiment)

Buckwheat is characterized by an increase in green matter throughout the entire period of vegetation. It is natural that with the increase of growth processes in plants, the process of forming a photosynthetic apparatus is accelerated.
In the vegetative experiment with buckwheat seed (Figure 8) we noted that the largest difference between the areas of the treated leaf surface and control plants was observed in the budding phase (Day 30) and was 11 %.

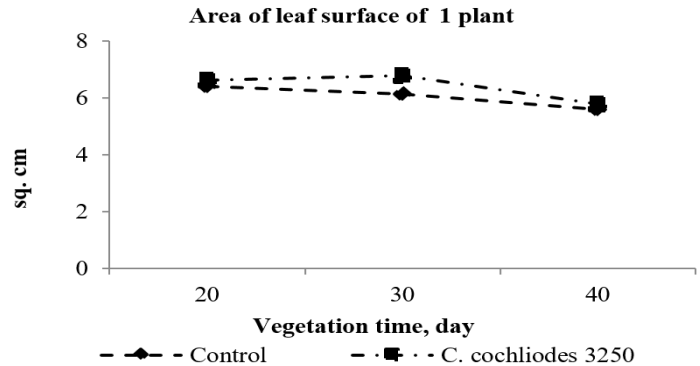

Figure 8 Area of buckwheat plants leaf surface under pre-sowing treatment of seeds by *C. cochliodes*3250 (vegetative experiment)

In addition, the formation of the endophytic association positively affected the productive performance of photosynthesis.
The obtained results showed an increase in the content of chlorophyll *a* in the leaves of inoculated buckwheat by 23 % on the Day 20 (branching phase), and by 6 % on the Day 30 (budding phase). The obtained results are presented in Figure 9.

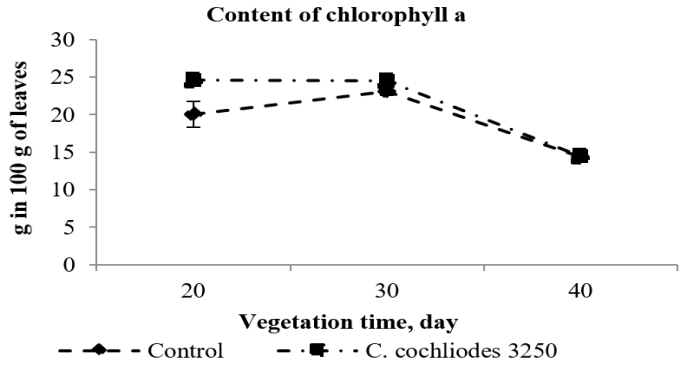

Figure 9 Content of chlorophyll *a* in the buckwheat plants leaves under pre-sowing treatment of seeds by *C. cochliodes*3250 (vegetative experiment)

The highest content of chlorophyll *b* was observed on the Day 30, as shown in Figure 10. Further, the content of chlorophylls *a* and *b* decreased and after Day 40 (flowering phase), no difference was found between the control and experimental plants.

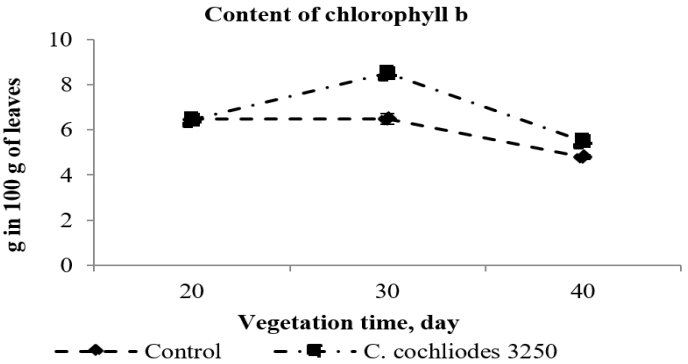

Figure 10 Content of chlorophyll *b* in the buckwheat plants leaves of under pre-sowing treatment of seeds by *C. cochliodes*3250 (vegetative experiment)

DISCUSSION

There is an evidence that soil fungi are able to survive on root surface and penetrate into root fibrils and rhizoderm cells forming endophytic associations (Chaves et al., 2009; Cevnik et al., 2000; Harrison, 2005; Hause, Fester, 2005; Ding et al., 2006; El-Zayat, 2008; Hata et al., 2010). Endophytism phenomenon of beneficial saprophyte fungi on plant root is a powerful factor for plant growth, metabolic processes activation and, as a result, subsequent macroorganism development. Microbial groups in root zone differ both in different species of plants (Innes et al., 2004; Batten et al., 2006) and within one species (Kowalchuk et al., 2006), as well as during ontogenesis of the individual plant (Weisskopf et al., 2006). The interaction between the microbiota and the root system is dynamic and based on co-evolutionary mechanisms (Broeckling et al., 2008).

As a result of our studying, we found out some interactions between plant and fungus symbiont provided in Figure 11

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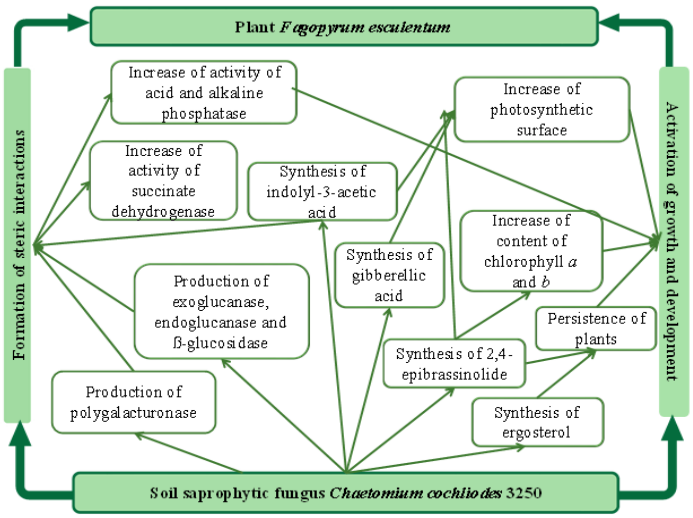

Figure 11 Functional relationship between micro- and macro-symbionts of the endophytic association *C. cochliodes* 3250/*Fagopyrum esculentum*

Various plant exudates and microbial metabolites such as enzymes and biologically active substances played an important role in formation of associations between soil fungi and plants (Yuan et al., 2010, Aly et al., 2010). Particularly it was established that the synthesis of physiologically active forms of auxins was typical for the majority of soil microorganisms forming assotiative relationships with plant (Iutynska, Ponomarenko, 2010). Metabolic products of *C. cochliodes* 3250 with auxin nature that we had found could stimulate cell growth by stretching, which was important for the conducting bundles formation, cambium and the additional roots formation.

The practical *C. cochliodes* 3250 usage was efficient due to the ability of its exogenous gibberellins to stimulate stem growth, increase the number of flowers and the size of the fruits, change the shape and size of flowers, accelerate seed germination, etc.

It is known that under the influence of phytohormonal substances synthesized by a microsymbiont, the photosynthetic activity of the plant improves, in particular, the amount of photosynthetic pigments increases (Smith, Read 2012). We also found that survival of saprotrophic fungus *C. cochliodes* 3250 living on a buckwheat root zone contributed to improved nutrition, activation of photosynthesis processes and increased of the total content of chlorophylls in plant leaves.

In plants adaptation to adverse environmental factors process, several cellular signalling systems, phytohormones and stress metabolites, are involved among which brassinosteroids draw attention of researchers. Brassinosteroids are physiologically active at very low concentrations (10^{-6} to 10^{-12}), which distinguishes them from other classes of phytohormones (Sakurai, Fujioka 1993). They can affect a wide range of morphological and physiological reactions: stimulate the growth of pollen tubes, change the shape of plant leaves, inhibit root growth, stimulate the synthesis of ethylene and increase the resistance of plants to abiotic stresses (Dragovoz et al., 2009; Tyuterov, 2015; Sharikova, 2001; Brass 1999). Previous studies showed that *C. cochliodes* 3250 was an active antagonist, and the mechanism of its action on pathogenic microorganisms was defined as direct competition (Nadkernychny et al., 1995). It can be assumed that the synthesis of 2,4-epibrassinolide also played a role in the competitive relations process, stimulating the growth of the plant root system and its potential for self-defence.

The presence of cholesterol in the cultural fluid may be an evidence of its role as a precursor in the synthesis of 2,4- epibrassinolide (Mandava, 1998). Plants use specific induced reactions that are activated only in response to contamination with a specific pathogen in order to protect from phytopathogens that overcome constitutional barriers. The high content of steroid derivatives indicates a high phytostimulating and protective activity of the fungus (Conrad, 2011). Receptor recognition of signalling molecules (ergosterol), produced by saprophytic fungus, promotes the development of induced systemic resistance of plants against phytopathogens of different origin (Olsson et al., 2003).

Some authors noted the presence of complex enzyme set in fungi -endophytes – cellulase, hemicellulose, polyphenol oxidase, polygalacturonase, which decompose plant tissues (Antonyak et al., 2013). Different species of fungi – producers of cellulose – differ in the formation way of the cellulolytic complexes individual components. The high cellulase activity of *C. cochliodes* 3250 stipulates its ability to penetrate the plant tissues.

Changes in hyphae membranes' structures of arbuscular fungi, when penetrating the intercellular space, were associated with the enzymes – pectinase (Gianinazzi-Pearson et al., 1981). That fact was confirmed by their biochemical detection in spores and free mycelium (Lobanok et al., 1988). Among the microscopic fungi capable to significantly produce the pectolytic enzymes were the representatives of the *Mucor, Rhizopus, Aspergillus, Penicillum, Fusarium, Alternaria* and other genera (Agrios, 2005; Avdeeva et al., 2010, Collmer, Keen, 1986). We have also detected polygalacturonase activity of the cultural fluid of *C. cochliodes* 3250, which determined the process of depolymerisation of the adhesive layer of pectin between the adjacent walls of plant cells.

Penetration into the root of plants and formation of appropriate mycorrhizal structures was accompanied with increased activity of the enzyme succinate dehydrogenase (SDH) (Sylvia, 1988; Hamel et al., 1990; van Aarle et al., 2005). The interdependence of symbiotic activity and SDH activity were confirmed by the histochemical staining of external (Hamel et al., 1990; Vierheiling, Ocompo, 1991) and internal (Macdonald, Lewis, 1978; Ocampo, Barea, 1985; Saito et al., 1993; Tawaraya et al., 1994) hyphae of arbuscular fungi for wheat, soybean and onions plants. SDH is a multifunctional enzyme complex, involved in the course of catabolic and anabolic processes. The enzyme catalyzed the oxidation of succinate to fumarate in the tricarboxylic acid cycle, and even the recovery of fumarate to succinatein in higher organisms (Eprintsev et al., 2007). At the same time, it was closely connected with the internal membrane of mitochondria and was a component of the Krebs cycle and the electron transport chain. Therefore its regulation was related to functioning of two processes of the cell oxidative metabolism (Pastore et al., 2001).

In the modern literature buckwheat is characterized as a classic non-mycorrhizal plant (Harley, Harley, 1987; Gai et al., 2006; Wang, Qiu, 2006). At the same time, published results of the molecular genetic analysis of fungi isolated from edaposphere and rhizosphere of culture, with DNA fragment sequences, were close to species of *Glomus*genus (Likar et al., 2008).

In our studies with common buckwheat, increase in SDH activity aligned with the active development of *C. cochliodes* 3250 on the roots of culture. Both signs indicate the formation of the endophytic association *C. cochliodes* 3250/common buckwheat.

The close interaction between plants and endophytic fungi that produce growth-stimulating substances significantly affects the structure of the root system. Thus, inoculation of plants with fungi of the genus *Trichoderma* was accompanied by an increase in the biomass of the roots, an increase in the intensity of the development of lateral roots and root fibrils (Contreras-Cornejo et al., 2009). Our results confirmed that the introduction of *C. cochliodes*3250 micromycetes into the root zone of buckwheat caused the basic physiological responses of plants to the formation of a symbiotic system: an increase in the total and active working surface of the roots.

It is known that the mycorrhization of plants increases the supply of phosphorus to the roots in 3-5 times (Smith, Read, 2008). For example, “mycorrhiza-specific” alkaline phosphatases (ALPs) was found in vacuoles of mature arbusculae, intercellular hyphae and in the hyphal glomus of Paris-type mycorrhiza (van Aarle et al., 2005). Previously, it was believed that ALP activity was limited by the internal structures of the root AM (Tisserant et al., 1993). However, appropriate enzyme was also found in free mycelium (Aono et al., 2004). The evaluation of alkaline phosphatase activity in mycorrhizal tissues was widely used as a parameter of the physiological efficiency of mycorrhizal infection. Functioning of acid phosphatase activity (AP) allowed maintaining the mobility of a large part of inorganic phosphorus during maturation of the crop (Turner, Plaxton, 2001). ALP and AP widespread in cells and played an important role in the metabolism of inorganic phosphates and cellular metabolism (Tabaldi et al., 2007; Mishra, Dubey, 2008). The evaluation of alkaline phosphatase activity in plant tissues colonized by mycorrhizal fungi is now widely used as a parameter of the physiological efficiency of mycorrhization. We revealed the increase in the activity of acid and alkaline phosphatase during the whole vegetation period in the buckwheat roots under the action of *C. cochliodes* 3250, indicating the efficiency of the formed symbiosis.

CONCLUSION

This study focused on features of the interaction between soil fungus *C. cochliodes* 3250 and buckwheat plants.

It was also shown for the first time that *C. cochliodes* 3250 was capable to produce 2,4-epibrassinolide (0.011 µg/mL of cultural fluid), which played an important role in plant resistance to pathogens and ergosterol (17.88 µg/mL of cultural fluid). In addition, the fungus synthesized phytohormonal substances: indolyl-acetic acid (8.7 µg/mL of cultural fluid) and gibberellic acid (56.4 µg/mL of cultural fluid). The indolyl 3-acetic acid and ergosterol synthesized by the fungus could be the main mediator molecules in the formation of *C. cochliodes* 3250 symbiotic systems with plants.

The exoglucanase, endoglucanase and β-glucosidase activity in the cultural fluid of *C. cochliodes* 3250 were established. *C. cochliodes* 3250 was capable to synthesize enzymes: exoglucanase (0.67 U/mL), endoglucanase (0.52 U/mL), β-glucosidase (1.02 U/mL) and polygalacturonase (2.95 U/mL), which was very important for its penetration into the plant root.

The action of fungi in the buckwheat roots increased the activity of succinate dehydrogenase (by 28%), acidic (by 326%) and alkaline (by 391%) phosphatase

during the whole vegetation period indicating the efficiency of the formed symbiosis.

It was found that pre-sowing treatment of buckwheat seeds by *C. cochliodes* 3250 fungus causes the basic physiological plants responses that are characteristic features for the symbiotic system formation. Namely they are increase of the total (by 145%) and active working (by 7%) root surface, increase of plant length (by 27%), leaf area (11%) and content of chlorophylls *a* and *b* (17%).

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