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Species of Fungi in the Root System of Woody Plants in Urban Plantations

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Abstract

The features of the species composition of microscopic fungi in the root system and rhizosphere soil of woody plants in connection with the level of soil contamination have not been studied sufficiently. This article presents the results of studying the species composition of fungi in the root system and soil of three species of woody plants (*Acer negundo* L., *Acer platanoides* L., and *Betula pendula* Roth.) growing in urban plantations of various ecological categories with different levels of heavy metal soil contamination. The study was carried out in a large industrial center of the Urals region, Izhevsk. When studying the species composition of fungi, microscopy and molecular genetic analysis were used. Isolates of endotrophic fungi from the root system of plants were isolated, and systematic membership was determined by molecular genetic analysis. The results showed that in soils with a high level of contamination, the DNA of endotrophic mycorrhiza-forming fungi was found in the roots of woody plants in a good living state.

Keywords: fungi, heavy metals, plantations, urban environment, resistance

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1. Introduction

Woody plants growing in cities, especially large ones, are convenient model objects for studying the mechanisms of plant adaptation to technogenic stress. In the underground sphere, such mechanisms are partially coupled with the interaction between plants and fungi such as mycorrhizal, pathogenic and saprotrophic interactions. In nature, the formation of mycorrhiza is the rule, and its absence is an exception. The most common type of mycorrhiza is arbuscular (AM). Many works, mainly looking at young agricultural and ornamental crops, are devoted to the physiological and biochemical interactions of plants with AM fungi (AMF) associated with the absorption, transportation and accumulation of nutrients. The involvement of AM in the metabolism and supply of plants with N, P, Cu and Zn is considered the most critical [1–4].

There are indications of changes in the pigmentary apparatus and mechanisms for increasing plant resistance (to drought, salinity and acidity of soils, temperature stress, the content of heavy metals in soils, exposure of pathogenic microorganisms and pest damage) upon symbiotic interaction with microscopic fungi [5–9].The purpose of our research was to study the species composition of microscopic fungi in the root system of woody plants and rhizosphere soils in urban plantations.

2. Methods

The research was carried out in the large industrial center Izhevskof the Urals region (the population is more than 630,000 people: industry, the transport network and the social infrastructure are developed, while the pollution level is high). The objects of research are woody plants (*Acer negundo* L., *Acer platanoides* L., *Betula pendula* Roth.), which account for about 70% of the green area of the city. The species studied grow in various structural and functional types of plantations with different levels of soil contamination: park plantations (the park named after S.M. Kirov, which has a low level of heavy metal concentration in the soil); tree plantings along the highway (Udmurtskaya Street) and plantations in the sanitary protection zone (SPZ) of the industrial enterprise OJSC Izhstal, which is the main polluter of the city (high level of heavy metal concentration in the soil); the plantings in the yard territories of the residential area 'Sever' and in the sanitary protective zone (SPZ) of the industrial enterprise Kerambloki (the average level of heavy metals concentration in the soil).

The record plants (5–10 trees of each species) were selected in each of the districts of the city. The selected plants were in a good living and middle-aged generative ontogenetic state [10]. In September, during the formation of suction root hairs and root system inoculation with mycorrhiza-forming fungi, samples of the root system and rhizosphere soil of each record plant were collected to study the fungal microflora. Isolation of endophytes from the woody plants' roots was carried out [11]. Samples of soil were stored at a temperature of +5–7°C. The roots were surface sterilized and dried at room temperature to an airily dry state, or subjected to rapid freezing with liquid nitrogen. DNA extraction was performed using the Ultra Clean Soil DNA Isolation Kit. For the PCR analysis, the primers ITS1-ITS4, ITS F-ITS A, ITS F-ITS B and FLR3-FLR4 were used. Then, the PCR product was cloned by inserting E. coli DNA *(Escherichia coli*), followed by plasmid preparation and PCR analysis using the M13 primer. After confirming the presence of fungi DNA in the plasmid, the samples were sent for sequencing (the 'Sangersequencing' method). To compare the results with known sequences, a list of annotated sequences present in the EMBL [12] and NSBI [13] databases were used. The extraction of DNA of roots, soil samples and isolated fungi was carried out at the Leibniz Institute of Vegetable and Decorative Cultures (Germany) and in the genetic laboratory of the Forestry Department of the Technical University of Zvolen (Slovakia).

The sampling of soils and their agrochemical analysis were carried out at the sites of the selection of plant samples. The analysis of the content of gross forms of heavy metals in soils was carried out at the Analytical Control Center (Accreditation Certificate No. POCCRU.0001.514685).

Table 1: The content of gross forms of heavy metals in soils of plantings of different ecological categories, mg/kg.

Element	Central Park Named after S. M. Kirov	'Sever'	SPZ of Kerambloki	OJSC Izhstal	Udmurtskaya Street
Cd	0.2 ± 0.1	0.05 ± 0.1	0.05 ± 0.1	*1.3 ± 0.3	0.05 ± 0.1
Mn	390.0 ± 82.2	895.0 ± 178.0	560.0 ± 77.0	*1822.0 ± 547.0	891.0 ± 187.0
Cu	3.8 ± 1.1	28.4 ± 8.5	20.3 ± 4.5	114.0 ± 34.0	85.0 ± 1.2
Ni	13.6 ± 4.0	18.9 ± 4.0	15.9 ± 4.0	46.4 ± 9.8	27.8 ± 5.6
Pb	11.6 ± 2.4	15.2 ± 4.5	13.2 ± 2.4	*103.0 ± 22.0	*43.6 ± 2.0
Zn	34.6 ± 7.3	51.9 ± 10.9	49.9 ± 8.9	*274.0 ± 82.0	94.0 ± 28.0

Source: Authors' own work.

Note. * The exceedance of the maximum permissible concentration.

3. Results

The soils in the park zone are referred to as natural because the transformation of the soil profile is no more than 50 cm and typical features are preserved. The sandy loam sod-podzol soils predominate there. The content of organic matter in these soils is 4.23%, the reaction of the soil solution is weakly acidic and close to neutral (pH$_{KCl}$ 5.83, pH$_{H2O}$ 6.70). On the whole, the soils are characterized by an average density; the field humidity is 17.08%. In the plantations growing in the district 'Sever' and in the sanitary protection zone of the enterprise Kerambloki, the soil profile has been transformed into urban soil. The soil solution was characterized by a slightly alkaline reaction, close to neutral. The soils had a normal density and humidity and a fairly high content of humus. In plantations of the SPZ of OJSC Izhstal, anthropogenic soils with a prevalence of hemozems (anthropogenically transformed soils) were recorded. The content of organic matter was high and accounted for 2.17%. The reaction of the soil solution

Table 2: Species composition of microscopic fungi in the roots of plants and soils, plants of different environmental categories (Izhevsk).

High level of concentration of heavy metals in the soil (plantations in SPZ of Izhstal and highway plantations Udmurtskaya Street HP)	Medium level of concentration of heavy metals in the soil (plantations of 'Sever' and plantations in SPZ of Keramblok)	Low level of concentration of heavy metals in the soil (Central Park named after S. M. Kirov)
Acer negundo L.		
Roots: *Dactylonectria alcacerensis* *Glomus intraradices** *Ilyonectria macrodidyma* *Inocybe umbrinella* *Tetracladium maxilliforme* **Soil:** *Ganoderma applanatum* *Geomyces pannorum* *Gibellulopsis nigrescens* *Glomus constrictum** *Inocybe umbrinella* *Meliniomyces bicolor* *Nectria gliocladioides* *Phialocepha lavirens* *Rhizophagus irregularis** *Trichocladium asperum* *Tuber rufum* (HP) **Isolated fungi from the roots:** *Ilyonectria pseudodestructans, I. crassa* *Leptodontidium orchidicola* (HP) *Phomopsis columnaris* *Trichoderma koningiopsis, T. asperellum*	**Roots:** *Cenococcum geophilum* **Soil:** *Ganoderma multipileum* *Nectria gliocladioides* *Phialocephala virens* *Rhizophagus irregularis** **Isolated fungi from the roots:** *Fusarium oxysporum,* *F. chlamydosporum,* *F. redolens* *Ilyonectria macrodidyma* *Neonectria ramulariae,* *N. macrodidyma,* *N. radicicola* *Penicillium citrinum,* *P. expansum,* *P. glabrum*	**Roots:** *Sarocladium kiliense* **Soil:** *Chaetomium globosum* *Sarocladium kiliense* **Isolated fungi from the roots:** *Absidia glauca* *Fusarium oxysporum,* *F. redolens,* *F. tricinctum,* *F. armeniacum,* *F. solani* *Neonectria macrodidyma,* *N. ramulariae,* *N. radicicola* *Penicillium canescens* *Phoma selaginellicola* *Phomopsis columnaris*
Betula pendula Roth.		
Soil: *Glomus claroideum**, *G. intraradices** *Retroconis fusiformis*(HP) *Tuber maculatum*(HP) **Isolated fungi from the roots:** *Oxyporus corticola*	**Soil:** *Glomus clarum**, *G. claroideum** *Russula exalbicans*	
Acer platanoides L.		
Soil: *Glomus clarum** (HP) *Glomus claroideum** (HP)	**Soil:** *Glomus* sp.*	**Roots:** *Fusarium equiseti* *Tetracladium maxilliforme* **Soil:** *Stachybotrys chartarum,* *S. chlorohalonata* **Isolated fungi from the roots:** *Fusarium tricinctum*
Source: Authors' own work.		
Note: * DNA of fungi that form arbuscular mycorrhiza.		

was neutral and alkaline (pH$_{KCl}$ 6.95; pH$_{H2O}$8.30). The soils were of average density and the field moisture was 13.61%. A complex of anthropogenic soils with a prevalence of stratozems (a mound on top of a natural profile) is revealed in plantings along the highway. The soil is characterized by pH_{KCl}6.97 and pH_{H2O}8.03, organic matter content is 2.29% and the average density and field humidity is 15.92%.

The results of soil analysis for the content of heavy metals are presented in Table 1. The identified species composition of microscopic fungi in plant roots and soils is presented in Table 2.

4. Conclusion

The results showed that the largest species diversity of microscopic fungi can be found in the soils and woody plants in the plantations of the sanitary protection zone of the enterprise Izhstal and in the plantings along the highway of UdmurtskayaStreet (*Betulapendula*, *Acer negundo, Acer platanoides*). No AMF was detected in the conventionally clean soils and roots of plants growing in the park zone.

We would like to note the presence of *Fusarium equiseti* fungi in the roots of plants. This endophyte is a modern object for studying the formation of plant resistance mechanisms based on interaction with fungi. For some plants, it provides protection against phytopathogenic fungi and viral infection while also increasing resistance to salts [14–17].

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