Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2020, 10 (3), 106-109, doi: 10.15421/2020_141

ORIGINAL ARTICLE

Species compositions of root rot agents of spring barley

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Received: 13.06.2020. Accepted: 10.07.2020

We studied the ratio of fungi genera in the affected root system of spring barley, the composition of pathogenic and comorbid mycobiota, which affected barley at different ontogeny stages. We discovered that the main root rot agents were *Fusarium* spp., *Bipolaris* spp., *Rhizoctonia* spp., *Pythium* spp., *Alternaria* spp., and the bacterium of *Pseudomonas* genera. In our research, 50.65% of *Fusarium* genera were taken at a sprouting stage from the affected root system, 54.25% were taken at tillering stage, and 56.25% were taken at milky-wax ripeness. The next abundant was *B. sorokiniana* fungus (20.6% of which were taken at sprouting stage, 18.4% at tillering stage, and 18.45% were taken at milky-wax ripeness. Less frequent were the representatives of *Rhizoctonia* genera – 19.45, 16.15, and 13.2% respectively. We suggested that while studying the pathogenic characteristics of root rot agents of spring barley, more attention need to be paid to their impact on the laboratory germination ability of seeds, the degree of the disease progression, biometric indices and plants persistence.

Keywords: Spring barley; Root Rot; Species composition of the agents; Correlation of fungi genera; Pathogenic mycobiota; Phases of plants ontogeny

Introduction

Spring barley is the fourth plant in the world as to the area of its cultivation after wheat, rice and maize, and it is the second in Europe. In Ukraine, it comes second after wheat as to the area under cultivation and to crop page. It is stipulated by its food, grain and feed value, by its commercial value, high yield capacity, tolerance to environmental conditions and to agrotechnology. However, root rot prevents the increase in the crop yield. The species composition of the agents and the peculiarities of their development are of great importance for the efficient root rots control of spring barley. Root rots of grain crops – it is a disease of roots and radical part of the wheat, barley and rye stalks, which is caused by one type or complex of *Fusarium*, *Helminthosporium*, *Ophiobulus, Pythium*, and *Rhizoctonia* genera (Korshunova et al., 1976; Nelson et al., 1983; Ashley et al., 2000).

The disease is manifested in roots affection, as well as in the affection of underground node, tillering node, stalk base and lower leaves. The affected roots and underground nodes get friable and break down when the plant is pulled from the soil. The tillering nodes get bulky and weak. The disease results in sprouting destruction, growth failure, and thin ears of the affected plants or in full decline of the productive stalks (Chulkina et al., 1974; Tepliakov & Tepliakova, 2003). Research aim was to study species composition of root rot agents of spring barley, to specify the correlation of fungi genera, which were taken from the affected root system of a crop, and to determine and specify the composition of pathogenic as well as of comorbid mycobiota, which affects the plants on different ontogeny stages of spring barley.

Materials and Methods

The dynamics of quantitative and qualitative composition of rhizosphere mycobiota of vegetative spring barley, was studied at the Field Research Station of National University of Life and Environmental Sciences of Ukraine during the vegetation periods of 2015–2017. Test samples were taken systematically regards the occurrence of disease symptoms by fungi etiology.

The laboratory research was conducted at Plant Pathology Department in a basic research laboratory of "Mycology and Plant Pathology". The micromycetes – facultative parasites were harvested and identified, the morpho-cultural characteristics of mycocenosis components of spring barley were studied. Herewith, we used the method of direct inoculation on the breeding grounds (Bilai, 1982) and the method of fungi accumulation in moist chambers (Naumov, 1937; Pidoplichko, 1977, 1988). The occurrence rate of some fungi species was defined by T.G. Mirchink method (Mirchink, 1976).

On a provisional basis, species can be called typical when the frequency of their occurrence is higher than 30%, sporadic – when the frequency is lower than 10%. Those species whose occurrence was lower than 30% but higher than 10%, were related to as "undefined" species (Mirchink, 1976). The drawn out roots were repeatedly washed by sterile water, dried in a few layers of sterile filtrating paper, put into Petri dish and then into a germination chamber at a temperature of 26 °C. The fungi growth observation was conducted during 24–48 hours on the following days of their growing. The identification of micromycetes species was done according to Khohriakov (1974) and Bilai (1977).

Results and Discussion

We revealed that the main root rot agents were *Fusarium* spp. (51.66%), *Bipolaris sorokiniana* (21.16%), *Rhizoctonia solani* (9.2%), *Rhizoctonia* spp. (5.56%), *Pythium* spp. (4.1%), *Alternaria alternata* (3.13%), *Alternaria* spp. (3.2%), and other fungi taxa (2.96%) and the bacteria of *Pseudomonas* genera (Table 1).

Table 1. Ratio of fungi taxa, taken from an affected root system of spring barley (sort Sebastian, 2015–2017).

Fungi taxa	Ratio of taxa in the affected root system of spring barley, %			
	2015	2016	2017	average
Pythium spp.	4.3	4.9	3.1	4.1
Bipolaris sorokiniana	14.0	20.8	25.7	21.16
Rhizoctonia spp.	6.6	5.2	4.9	5.56
Rhizoctonia solani	11.3	10.0	6.3	9.2
Fusarium spp.	53.2	50.2	51.6	51.66
Alternaria spp.	4.7	2.1	2.8	3.2
Alternaria alternata	2.8	3.0	3.6	3.13
Other genera	3.1	3.8	2.0	2.96

We concluded that *Fusarium* genera were the most frequent in the affected root system of spring barley within 2015–2017. In 2015, they amounted up to 53.2%, in 2016 – 50.2%, and in 2017 – up to 51.6% respectively. The ratio of *Bipolaris sorokiniana* to the total amount of isolates was the second after *Fusarium* spp. In 2015, they were reached 14.0%, in 2016 – 20.8%, and in 2017 – 25.7%. We accepted that the development of root rots in different crops is caused by many soil micromycetes, that is why it is necessary to determine and specify the composition of the pathogenic and comorbid mycobiota, which affects the plants at different ontogeny phases. In our research, main share of *Fusarium* genera (50.65%) were taken at a sprouting stage of the affected root system, 54.25% was taken at tillering stage, and 56.25% - at milky-wax ripeness – 56.25% (Figure 1a, Figure 2b and Table 1).

Table 2. Microbiological analysis of the affected roots of spring barley (sort Sebastian, 2015–2017).

Fungi genera	Number of plants with the fungi, %		
	Sprouting	Tillering	Milky-wax ripeness
Pythium spp.	4.2	4.5	4.35
Bipolaris spp.	20.6	18.4	18.45
Fusarium spp.	50.65	54.25	56.25
Rhizoctonia spp.	19.45	16.15	13.2
Alternaria spp.	3.95	4.2	4.15
Other genera	1.15	2.5	3.6

Second frequent regards occurrence was *Bipolaris fungus* (20.6% were registered at sprouting stage, 18.4% – at tillering stage, and 18.45% – at milky-wax ripeness, Figure 2a). Much less frequent were the representatives of *Rhizoctonia* genera – 19.45, 16.15, and 13.2% respectively (Figure 2a and Figure 3). They were isolated together with the fusaria. A few representatives of different genera (Table 2) were often taken from the affected root system. The fungi of *Pythium* and *Alternaria* genera (Figure 1b and Figure 3) were identified in lower amounts – 4.2, 4.5, 4.35 and 3.95, 4.2, and 4.15%, respectively.

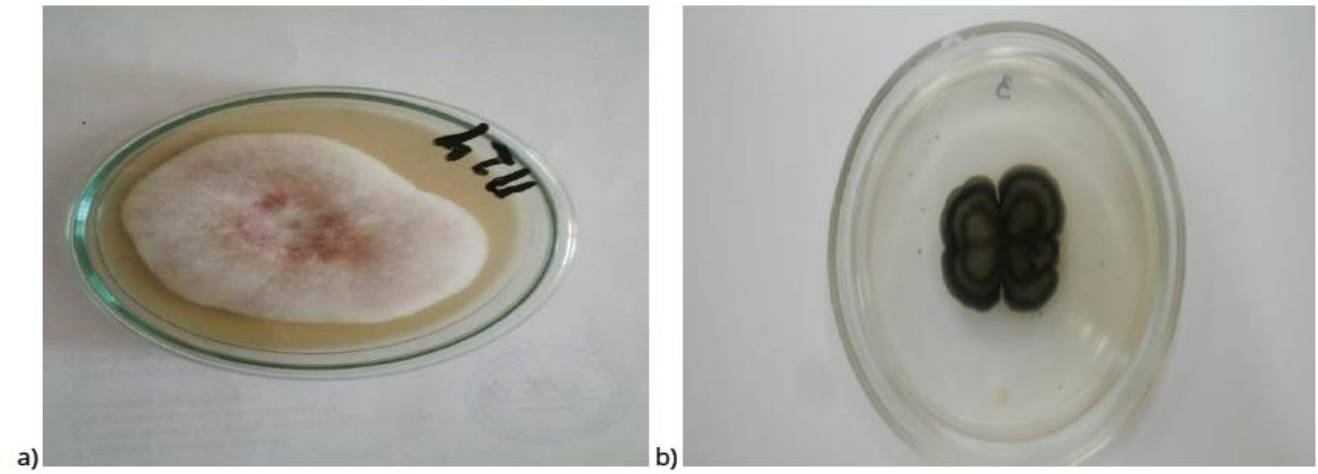

Figure 1. Fungus colony of *Fusarium* (a) and *Alternaria* (b) genera isolated from the affected root system of spring barley (Czapek's medium, 7th day of cultivation).

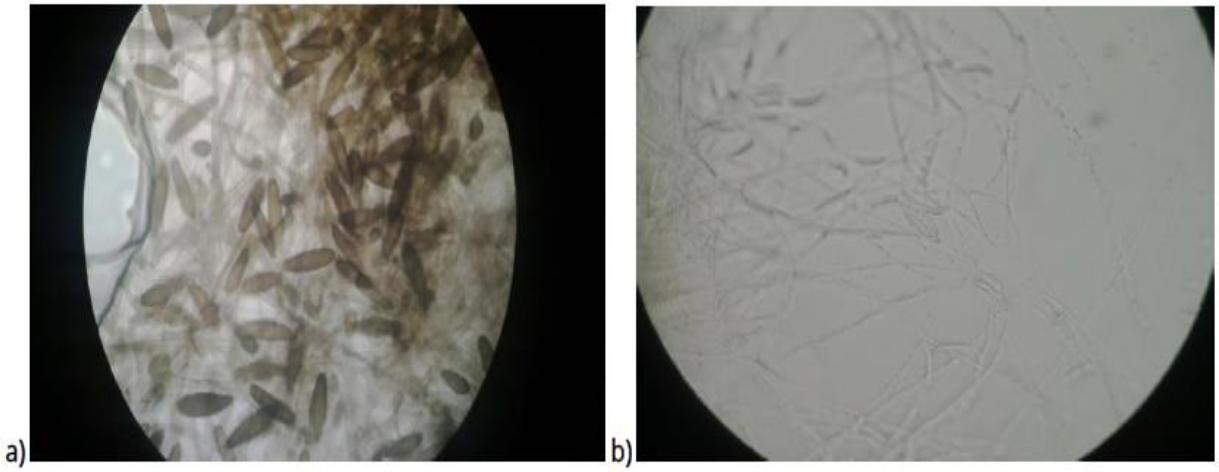

Figure 2. *Bipolaris sorokiniana* (a) and *Fusarium oxysporum* (b), 40x.

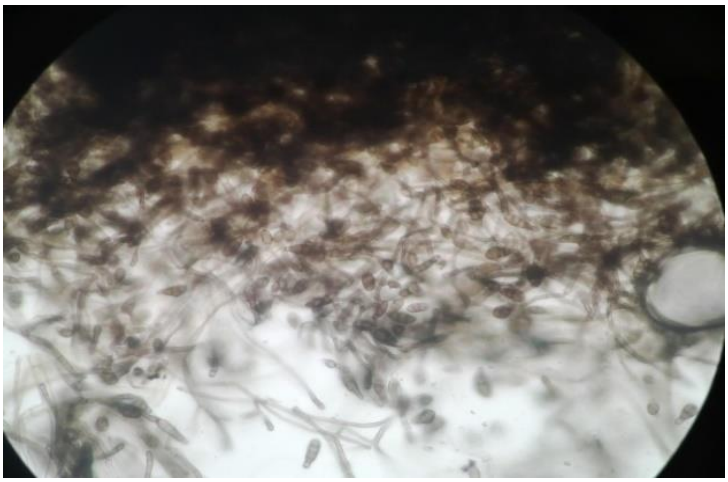

Figure 3. *Alternaria alternata*, 40x.

While studying the pathogenic characteristics of root rots agents of spring barley, we also registered their impact on laboratory seeds sprouting and disease rate progression, biometrical indices and plants persistency. In laboratory conditions and under the influence of *Fusarium* species the seeds sprouting in *F. oxysporum* was decreased by 76.25%, in *F. solani* – by 73.75%, in *F. graminearum* – by 67.5%, and in *F. avenaceum* – by 65%; this was 80.0% in the control (Table 3).

Table 3. Pathogenicity of the spring barley isolates, affected by the root rot (sort Sebastian, laboratory experiment, 2016–2017).

Variant	Laboratory seeds sprouting, %	Sprouting		Plant heights	
		Affected plants, %	Disease progression, %	cm	%
Control without fungi introducing	80.0	12.0	4.75	18.7	100
Fusarium graminearum	26.0	61.0	22.25	10.3	55.1
Fusarium avenaceum	28.0	59.0	21.5	10.7	57.2
Fusarium oxysporum	19.0	75.0	49.0	8.2	43.8
Fusarium solani	21.0	71.0	46.75	8.6	45.9

The ratio of spring barley pathogenicity without the introducing of fungi was 12%, under the action of *F. oxysporum* – 75.0%, *F. solani* – 71.0%, *F. graminearum* – 61.0%, and under the action of *F. avenaceum* – 59.0%. The disease progression increased by 10.3, 9.8, 4.7, and 4.5 times respectively; the plant heights was less by 56.2, 54.1, 43.78, and 44.9%. These indices in the control were 4.75% and 18.7 cm (Table 3).

Conclusion

We established that rhizosphere mycobiota of the root system of spring barley is represented by 10 fungi genera. The most spread root rot agents of spring barley were *Fusarium* spp. (51.6%), *Bipolaris sorokiniana* (25.7%), *Rhizoctonia solani* (6.3%) *Rhizoctonia* spp. (4.9%), *Pythium* spp. (3.1%), *Alternaria alternata* (3.6%), and *Alternaria* spp. (2.8%). Other genera (2.0%) were represented by *Mucor* spp., *Penicillium* spp. and *Aspergillius* spp. The species composition of agents was not constant and depended on the meteorological conditions and plant development phase. We registered that the most pathogenic agents were *F. oxysporum* and *F. solani.* In laboratory conditions, the seeds sprouting decreased in *F. oxysporum* by 76.25%, in *F. solani* – by 73.75% under the influence of these fungi; the disease progression increased by 10.3 and 9.8 times, the plants height was by

56.2% and 54.1% lower. The protection of spring barley against these agents would allow to decrease the intensity of root rots development and to increase the barley yield.

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Citation:

Gentosh, D.T., Kyryk, M.M., Gentosh, I.D., Pikovskyi, MY., Polozhenets, V.M., Stankevych, S.V., Nemerytska, L.V., Zhuravska, I.A., Zabrodina, I.V., Zhukova, L.V. (2020). Species compositions of root rot agents of spring barley. *Ukrainian Journal of Ecology, 10* (3), 106-109.